THE TIME OF ACTION OF THE GENE *ANTENNALESS* AND ITS EFFECT ON THE DEVELOPMENT OF THE CEPHALIC COMPLEX OF *DROSOPHILA MELANOGASTER*

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(With Four Text-figures)

1. INTRODUCTION

The mutant gene *antennaless* first appeared in a rough-eyed stock of *Drosophila melanogaster* belonging to Dr Cecil Gordon. Under suitable environmental conditions, it gives rise to flies with no antennae (A_0). In other circumstances, flies of the same pure line may have two normal antennae (A_2) or one antenna (A_1). Intermediate types are rare. Some of the external conditions which influence exhibition of the gene have been examined by Gordon & Sang (1941), who have shown that it is possible to alter the proportion of the three phenotypes formed in a single culture by rearing the larvae at different temperatures. This has enabled them to determine the *temperature effective period* (T.E.P.) of the gene. The critical phase, found to occur about 3 days after hatching in this instance, is commonly taken to be identical with the time of action of the gene (Goldschmidt, 1938). The present investigation is an attempt (*a*) to find if the time of action so determined can be confirmed by other methods, and (*b*) to examine the general effects on a single organ produced by a single gene particularly sensitive to environmental conditions.

We have employed morphological examination, measurement of the organ affected, and the use of new culture media for the determination of the time of action of the gene. A comparison of the development and of growth rates of the frontal sacs in the different phenotypes of genotypically identical larvae gives a measure of the nature and time of divergence of *antennaless* from the normal. Such observations can be made entirely on A_1 larvae. Further, by successive implantation of larvae into media known to encourage high and low levels of gene exhibition, we can find the time at which the developing organ becomes autonomous with respect to the environment in question.

2. MATERIALS AND METHODS

(*a*) *Egg collection and sterilization technique.* The method of egg collection and sterilization is identical with that used by Gordon & Sang (1941) and Robertson & Sang (1944), except that we used twice the usual amount of $HgCl_2$, since the ordinary prescription (White's fluid) was inadequate to ensure sterility in all cultures. Larvae aged less than 8 hr. grew in sterile media described below.

(*b*) *Sterile media.* The three media used in the experiments to be described had the following compositions:

*M_1	Dried brewers' yeast	$\frac{1}{3}$ g.
	Sieved sawdust...	$\frac{2}{3}$ g.
	Pearl S 101 salt solution + 3 % dextrose	4 ml.
*M_2	'Perolin' bakers' yeast...	$\frac{1}{3}$ g.
	Sieved sawdust...	$\frac{2}{3}$ g.
	Pearl S 101 salt solution + 3 % dextrose	4 ml.
M_3	Dried brewers' yeast	$\frac{1}{3}$ g.
	Sieved sawdust...	$\frac{2}{3}$ g.
	Pearl S 101 salt solution + 24 % dextrose	4 ml.

* See Gordon & Sang (1941).

The vials containing the medium were autoclaved for 20 min. at 20 lb. pressure, the larvae being then transferred to them by means of sterilized platinum spoons.

(*c*) *Indices of gene exhibition.* Gordon & Sang (1941) made use of three indices of exhibition (α, β, γ) to express the proportions of the various phenotypes found in a given culture. If A_0, A_1, A_2 stand for the number of homozygous flies of *antennaless* stock having no antennae, one antenna or two antennae respectively, then

$$\alpha = \frac{A_0 + A_1}{A_0 + A_1 + A_2}, \quad \beta = \tfrac{1}{2}(\alpha + \gamma), \quad \gamma = \frac{A_0}{A_1 + A_2 + A_0}.$$

These same indices will again be used as a measure of the characteristics of a culture.

(*d*) *Histological technique.* Larvae removed from the standard sterile medium (M_1) by flotation in a sterile salt solution of high specific gravity, were fixed in Kahle's fluid for 1 hr. at 60° C. Dehydration prior to embedding in wax was then carried out according to the following routine already described in D.I.S. (1941).

1. 70 % alcohol	Overnight
2. 30 % alcohol	1 hr.
3. 45 ml. 45 % alcohol + 5 ml. normal butyl alcohol ...	2 hr.
4. 42 ml. 62 % alcohol + 8 ml. normal butyl alcohol ...	2 hr.
5. 32 ml. 77 % alcohol + 17 ml. normal butyl alcohol ...	4 hr.

6. 22 ml. 90% alcohol + 27 ml. normal butyl alcohol	...	Overnight
7. 12 ml. 90% alcohol + 37 ml. normal butyl alcohol	...	4 hr.
8. Normal butyl alcohol	...	Overnight
9. Normal butyl alcohol	...	Overnight or till required

Sections were stained in Mallory's phosphotungstic acid haematoxylin.

(*e*) *Measurement technique.* In order to measure the growth of the antenna buds, larvae of the required age were cleaned in a saline solution and dissected under a binocular. The head was pulled away from the body, and the frontal sacs (eye bud + antenna bud) separated off. After further dissection such preparations were fixed and stained in aceto-carmine and mounted in Farrant's medium. When a series of preparations had been collected they were each projected, at constant magnification, on to a ground glass screen and photographed on to Kodak R.P. 30 oscillograph paper. The image of the antenna bud so obtained was cut out and the weight of the paper determined with a torsion microbalance. By reference to a photograph of 100 $\frac{1}{100}$ sq. mm. squares of a haematocrit similarly photographed the area represented by 1 mg. of paper was determined.

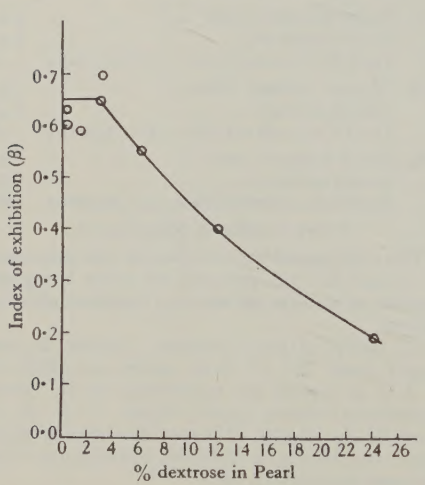

Fig. 1. Effect of dextrose on the exhibition of the gene *antennaless*.

(*f*) *Environmental control of gene exhibition.* In the course of experiments on the effect of environment on gene exhibition, we found that high concentrations of dextrose greatly lowered gene exhibition (Fig. 1). This suggested that we could obtain a measure of the time of action of the gene by transferring larvae from a medium of low to one of high dextrose content. Accordingly, larvae of definite age were removed under sterile conditions from the low-sugar content medium (M_1) on to sterile agar slabs. Traces of the salt flotation solution were removed from the larvae, as they crawled over these slabs. The larvae were then seeded on to a medium of high dextrose content (M_3). Controls were removed from the incubator while transfers were made. The sterility of all vials was tested at the time of transfer and at the end of the experiment. No infection occurred.

3. DEVELOPMENT OF THE CEPHALIC COMPLEX IN A_1 FLIES

Robertson (1936) and Chen (1929) have described the normal development of the frontal sacs of *D. melanogaster*. Here we shall only describe the relevant details of the development of a half-antennaless individual. For this purpose we assume that larvae showing lateral asymmetry of the developing buds would have given rise to half-antennaless imagos.

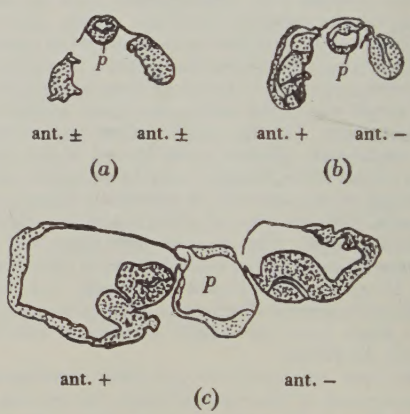

Fig. 2. Stages in development of the antenna buds of a half-antennaless larva: (*a*) about 70 hr., (*b*) 96 hr., (*c*) pupa just prior to fusion of frontal sacs. *ant.* + = normal antenna bud. *ant.* − = antennaless bud. *p* = pharynx.

The left and right antennal buds are not different in appearance up to about 70 hr. from the time of larval hatching. At this stage, both appear in cross-section as pear-shaped thickenings of the frontal sacs hanging down on either side of the pharynx (Fig. 2 *a*). The eye bud is not yet clearly marked off from the antenna bud and no folding or segmentation has yet taken place. The sacs are connected down their length by a thin sheet of tissue.

The effective developmental changes are visible after this and continue during the next 30 hr. Fig. 2 *b* shows the buds of a 96 hr. A_1 larva. There are now two differences between the wild type (*ant.* +) and antennaless (*ant.* −) sides. First, the normal bud (*ant.* +) is much larger than its fellow, the antennaless bud (*ant.* −). Models prepared at this stage show that

the size difference mainly affects the diameter and not the length of the bud. This is what would be expected in view of how the antenna is formed (Chen, 1929). Second, the antennaless bud is unsegmented, while the wild type bud is segmented. Failure to segment in antennaless (*ant.*−) results in the absence of the 'nipple' found in the normal buds. Hence, the gene *antennaless* acts both on the normal growth of the bud, and on its differentiation.

At about 100 hr., growth of the antennaless (*ant.*−) bud appears to cease. Shortly before pupation, the anterior wall of the normal sac is found to be much thicker than that of the antennaless one. This segmented thickening is the antenna bud proper. Occasionally, the eye bud on the antennaless side also shows some diminution. This tallies with the observation that reduction of the imaginal eye sometimes occurs on the antennaless side.

As regards the cephalic complex, the prepupal phase is marked by medial fusion of the frontal sacs. This is true of all three phenotypes, and the process is completed about 12 hr. after pupation. Fig. 2 *c* shows a cross-section of an A_1 pupa just prior to this fusion. The antenna bud now consists of a segmented thickening about half way down the frontal sac. The antennaless sac is only slightly thickened. Eventually the frontal sacs are everted to form the head of the adult.

4. GROWTH RATE OF THE NORMAL AND ANTENNALESS CEPHALIC COMPLEX

Fig. 3 and Table 1 show the mean growth rates of *ant.*+ and *ant.*− buds. The normal growth curve was determined on two media (M_1 and M_2); the growth rate of the antennaless *anlage* was determined on M_1 only.

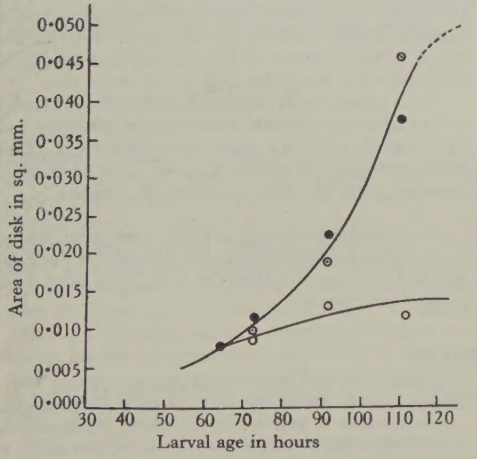

Fig. 3. Growth curves for normal (*ant.*+) and antennaless (*ant.*−) antenna buds. −●− = normal on M_1. −⊙− = normal on M_2. −○− = antennaless (*ant.*−) on M_1.

The graphs show that up to about 65 hr. the growth rate of normal (*ant.*+) and antennaless (*ant.*−) imaginal disks is approximately the same. Thereafter, the normal disk grows and differentiates rapidly following an exponential curve, while the antennaless disk grows very slowly. That is, the general conclusions reached by histological examination are confirmed. The time of separation of the growth curves gives an independent measure of the time of action

Table 1

Larval age hr.	Growth medium	Area of antenna disk (sq. mm.)	
		A_2	A_0
64½	M_1	0·0076	0·0076
73½	M_1	0·0115	0·0088
91¾	M_1	0·0223	0·0130
111½	M_1	0·0373	0·0118
72¾	M_2	0·0095	—
91¾	M_2	0·0189	—
111¾	M_2	0·0455	—

of the *antennaless* gene. Before 65 hr. we have no indications that the growth of the frontal sacs is in any way affected by the presence of this gene, but provided specific external influences, to be discussed later, do not intervene, growth slows down rapidly. In the presence of such substances, as in the normal wild type, growth continues exponentially.

5. ENVIRONMENTAL CONTROL OF GENE EXHIBITION

Fig. 4 and Table 2 show the results of transferring larvae of various ages from a sterile medium of low dextrose content (M_1) to a sterile medium of high

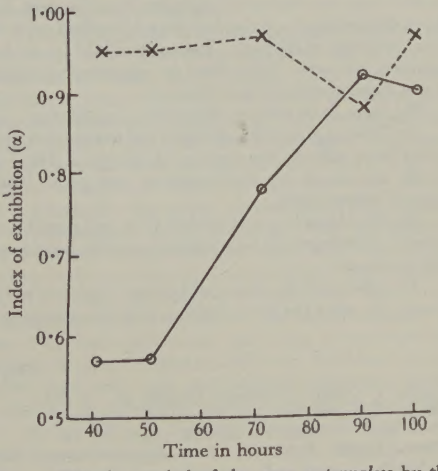

Fig. 4. Effective period of the gene *antennaless* by the dextrose technique. -- × -- = control cultures. −⊖− = experimental cultures.

dextrose content (M_3). The upper curve (--×--) shows indices for flies of the control group, grown throughout on M_1. The lower curve (—⊙—) shows indices for larvae transferred at 42, 51, 72, 90 and 100 hr. from M_1 to M_3. These two curves show that exhibition can be modified by alterations of the environment at all times prior to 60 hr. Between 60 and 70 hr., the proportion of animals which can be

Table 2

Age at transfer hr.	Index of exhibition					
	Control			Experimental		
	α	β	γ	α	β	γ
42	0·95	0·69	0·42	0·57	0·32	0·07
51	0·95	0·65	0·34	0·57	0·32	0·06
72	0·97	0·87	0·77	0·78	0·53	0·27
90	0·88	0·63	0·37	0·92	0·76	0·60
100	0·97	0·71	0·44	0·90	0·72	0·54

so affected declines rapidly. That is, most 75 hr. old larvae, at 25° C. on the particular medium (M_1) used, have reached a stage at which antenna determination is complete. Thereafter, antenna formation is no longer influenced by nutritional factors. On the basis of these experiments we may say that the effect of the gene can no longer be modified about 80 hr. after hatching.

DISCUSSION AND CONCLUSIONS

Many workers have studied the role of the gene in determining developmental processes (see Waddington, Beadle, Goldschmidt, etc.). In a number of instances the T.E.P., i.e. period during which the gene action may be altered by temperature changes, has been determined and this has been identified with the time of action of the gene. Recently, Child (1940) has pointed out some difficulties in interpreting results from experiments of this kind.

We have been able to determine two limits:

(*a*) The earliest time at which the *antennaless* gene manifestly affects the course of antennal development, as shown by measurement and gross histological examination.

(*b*) The latest time at which it is still possible to modify development by means of specific nutritional substances.

The effects of the gene can be first discerned about 70 ± 5 hr. after the larva hatches and this agrees well with the T.E.P. found by Gordon & Sang (1941). It is impossible to modify the development after about 80 hr. Hence, our two limits (*a*) and (*b*) lie very close together and are probably not more than 12 hr. apart for an individual larva.

On the assumption that this critical phase represents the time of action of the gene, it would be reasonable to say that this also corresponds to the T.E.P. However, the first assumption is an arbitrary one, since we have no means of knowing how early in development the gene actually becomes physiologically active. This initial action need not necessarily result in specific physiological changes in the developing organ (e.g. the tissue may not yet be 'competent') and for this reason it may be better to talk of the 'effective period' instead of the 'time of action of the gene'.

SUMMARY

A. The development of the antenna buds of the mutant *antennaless* of *D. melanogaster* has been examined and it is shown that:

(1) Prior to about 70 hr., no histological difference is detectable as between normal and abnormal *anlagen*.

(2) Normal antenna buds continue to grow exponentially after 70 hr.; abnormal buds scarcely grow at all.

(3) The abnormal bud is also distinguished from wild type by its failure to segment.

(4) Both normal (*ant.* +) and antennaless (*ant.* −) frontal sacs are everted normally in the pupa.

B. The period during which the course of development of antennaless buds of the same genotype could be modified by specific nutritional substances was also determined. It is shown that media containing a high concentration of dextrose will induce a lowered exhibition. By transferring larvae from normal media to media of high dextrose content, it is found that the course of development of the antenna bud cannot be modified after about the 80th hr. of larval life.

These experiments suggest that the *effective period* of the gene is relatively short and is probably not more than 12 hr. for each individual larva. This period corresponds to the T.E.P., but is not necessarily identical with 'the time of action of the gene'.

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REFERENCES

CHEN, T. (1929). *J. Morph. Physiol.* **47**, 136–87.
CHILD, GEORGE (1940). *Genetics*, **25**, 354–65.
Drosophila Information Service, 1941, vol. 12.
GOLDSCHMIDT, R. (1938). *Physiological Genetics*. New York.
GORDON, C. & SANG, J. H. (1941). *Proc. Roy. Soc.* B, **130**, 151–84.
ROBERTSON, C. W. (1936). *J. Morph.* **59**, 351–99.
ROBERTSON, F. W. & SANG, J. H. (1944). Ecological determinants of population growth. I. Fecundity of adult flies. (In the Press.)
WADDINGTON, C. H. (1939). *Introduction to Modern Genetics*.

THE INFLUENCE OF VARIOUS PHYSICAL AND BIOLOGICAL FACTORS OF THE ENVIRONMENT ON HONEYBEE ACTIVITY. AN EXAMINATION OF THE RELATIONSHIP BETWEEN ACTIVITY AND NECTAR CONCENTRATION AND ABUNDANCE

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(With Five Text-figures)

INTRODUCTION

Although there are frequent references in the literature to honeybees visiting one species of 'bee-plant' and neglecting other species growing near at hand, even though the latter may be attracting many bees in another district a few miles away, little has been done to determine the factors which affect the working of nectar-producing flowers by honeybees and other pollinating insects. It frequently happens, for instance, that honeybees placed in orchards to pollinate the fruit trees will neglect the flowers of the latter in favour of the flowers of weeds such as the dandelion (*Taraxacum Dens-leonis*) growing beneath the trees. Similarly bees will sometimes visit the flowers of some cover or fodder crop rather than the flowers of the fruit or seed crop growing nearby.

The responses of honeybees to the relative attractiveness of various species of plants in visiting their flowers in search of nectar and pollen have been under observation for a number of years in the neighbourhood of Harpenden, Hertfordshire. In the present paper two of the factors which play an important part in influencing these responses are examined. These are nectar abundance and nectar concentration.

METHODS

Collection of nectar samples for analysis

Samples of nectar were obtained from the flowers in one of two ways. One method was to obtain the nectar directly from the surfaces of the nectaries of the flowers by means of a small pipette. For this purpose fine capillary tubes of 'pyrex' glass each about 4 in. long were employed; the nectar, when one end of the capillary tube was brought into contact with it, entering the tube by capillary attraction. Although this method was extremely valuable as a check on the other method employed, it was very tedious and, except in the case of heavy 'honey flows' of lime and plum, it took an hour or more to collect 1 ml. of nectar.

The second and most frequently used method was to catch a number of honeybees or, more rarely, bumblebees found working on the flowers under examination and to obtain the nectar that they had collected by exposure and dissection of the honey-stomach. This method, although very much quicker than the former, might be considered as being open to serious objections, as the honey-stomachs of the bees concerned might contain nectar collected from a number of different species of plants. Fortunately, however, this error is unlikely to occur except on very rare occasions, since it is now a well-recognized fact that a honeybee on any one foraging trip only visits the flowers of one species of plant and, indeed, confines its attention to a very small area of the plants concerned (Butler, Jeffree & Kalmus, 1943). Exceptions to this rule do undoubtedly occur, but there is good reason to believe that they are very rare (Butler, 1941). More error arising from inconstancy to a particular species of plant on any one foraging trip is likely to occur in the case of bumblebees which do tend to wander from one species to another to a far greater extent than is the case with the honeybee. For this reason, therefore, nectar was only taken from bumblebees when it could not be obtained satisfactorily in any other way.

In order to avoid very much greater errors it was necessary when collecting honeybees for nectar extraction to make quite certain that only nectar gatherers were collected, not pollen gatherers, and that only those gathering nectar from flowers growing near (up to $\frac{1}{4}$ mile) their hive were taken. Collection of nectar from bees also had to be confined to good, warm, flying days when the bees were very active. The reason for the above restrictions of this method of nectar collection lies in the fact that, particularly at the lower temperatures at which flight occurs and when the bee is working at some distance from its hive or is a pollen gatherer, it carries out of the hive a certain amount of honey in the honey-stomach to serve as fuel until nectar gathering is commenced. The amount of honey carried from the hive is probably correlated with the weather conditions at the time and the distance that the bee has to fly before it reaches its foraging site, but no accurate determinations have, so far as is known, been made to date. It was found, however, that for short flights to the foraging ground on good flying days very little

honey was carried and that the inaccuracies thus introduced were too small to affect the results materially. Similar results were obtained by Vansell (1942) in California and Oregon.

One other possible objection to this method of collecting nectar samples should perhaps be mentioned. It is still a fairly prevalent idea that bees extract and get rid of water from nectar contained in the honey-stomach whilst carrying it to the hive. However, Park (1933) disproved this theory and showed that nectar undergoes no concentration whilst being transported in the honey-stomach of the bee.

Analysis of nectar samples

In order to determine the total carbohydrate in the small samples of nectar collected, use was made of a modification of the Tillmans-Phillipi method described by Pirie (1936). Each 1 ml. sample of nectar was divided into two equal parts. To each of these 2 ml. of orcin* reagent (0·2% of orcin made up in 66% sulphuric acid) were added and the whole then diluted to 10 ml. with distilled water. This mixture was heated in a briskly boiling water-bath for 15 min., cooled, diluted to 50 ml., again cooled and a 10 ml. sample compared in a Lovibond Tintometer with a standard containing a known quantity of sucrose which had been treated in a similar manner. In this way two determinations of the total carbohydrate content of each 1 ml. sample of nectar were made, the mean of these two estimations being taken as the final figure.

RESULTS

Effect of nectar quantity on visits by honeybees

Great difficulty was experienced in all attempts to measure the amount of nectar present in the flowers at any given time, and attempts to obtain direct measurements of the amount of nectar per flower had to be abandoned. Attempts were then made to determine the quantity of nectar present in the flowers of one particular species of plant at a time by noting the length of time that a particular bee working on the species of plant under examination spent in the field on any one foraging expedition. The bee was marked with a spot of paint on the dorsal surface of the thorax for purposes of identification. A check that the bee under observation had in fact been working the species of plant whose nectar abundance was being studied was afforded by identification of the pollen which the bee was carrying when it returned to its hive at the end of a foraging expedition. A small number of observations of this type were made when the bees were working dandelion, cherry, plum and other plants. Such observations were abandoned, however, since it became apparent that the length of time that an individual bee spent in the field was almost certainly influenced by a number of factors besides that of nectar abundance. Great differences in the length of time spent by individual bees on any one foraging expedition were noted even when, as was usual, the observations were made upon good flying days. On these days climatic conditions were such that factors which have been shown to influence honeybee activity greatly (such as light intensity, Butler & Finney, 1942) were not considered to be varying sufficiently to reduce the length of time spent by the bees in the field. Furthermore, it seems probable that the abundance of nectar in the flowers of two or more species of plants cannot be measured and compared accurately in this way since the structure of their respective flowers would render the nectar more or less easily accessible to bees.

So far as can be determined no accurate measurements of the volume of nectar contained in a flower in relation to honeybee activity have as yet been made. However, there is some evidence that nectar quantity has some effect in determining upon which species of plant the greatest number of honeybees will be found at any given time. Von Frisch (1938) has shown that honeybees communicate to one another the abundance and concentration of nectar of particular plant species by means of the 'wag-tail' dance and the scent of the flower concerned on returning to the hive after foraging. He has further shown that as the quantity of the nectar of a given species of plant becomes smaller, irrespective of its sugar concentration, so the number of bees working the flowers of that plant which dance on returning to the hive is reduced. Finally, when the nectar is almost exhausted, no bees are dancing and few new foraging bees are attracted to that source of nectar supply, although the bees that have been working the flowers of the plant concerned continue to work them until they no longer contain any nectar. These observations have to a large extent been confirmed by recent field work of Butler *et al.* (1943), who showed that once a bee becomes 'fixed' to a particular foraging site (4–5 yd. in diam.) on a particular flowering crop it is very loathe to give up visiting the site concerned and in fact continues to visit it for some hours, often even for some days, after the supply of nectar has become completely exhausted. Few, if any, new foraging bees, however, are attracted to such sites once the nectar supply becomes depleted. This is clearly shown in Figs. 1 and 2, which give the results of two typical experiments designed to study the effect of a reduction in the quantity of 'nectar' (20% sucrose syrup), followed by an increase in quantity, upon the number of honeybees visiting the source of 'nectar'. Ten Petri dishes each 4 in. in diameter and about one-third full of well-washed silver sand were placed early one morning on a table in an apiary. The sand in each dish was thoroughly moistened with syrup containing a trace of oil of lavender and observation kept on the dishes. As soon as the first bee had found the dishes, fed, and left the table to return to her hive counts of the total number of honeybees collecting

* The orcin was supplied by Messrs Hopkins and Williams and the orcin reagent freshly prepared for each day's batch of estimations.

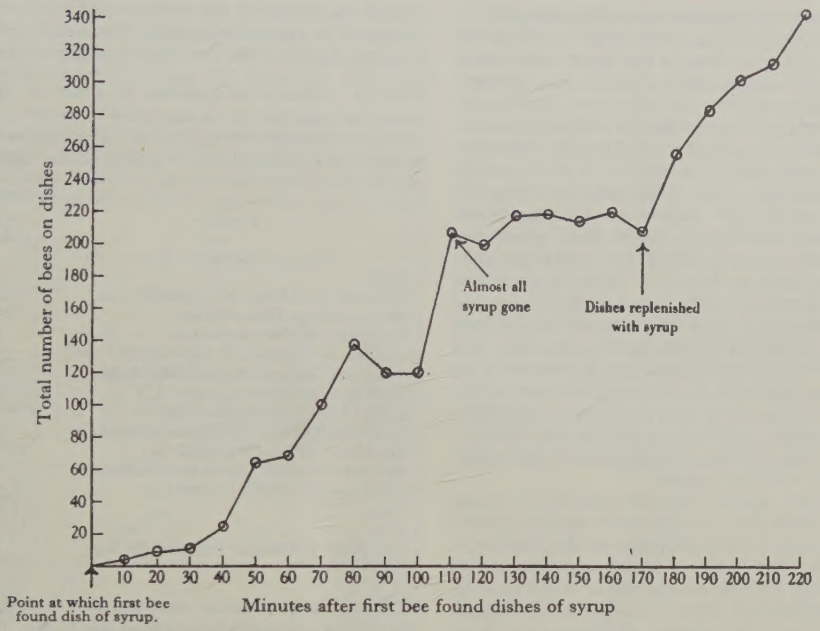

Fig. 1. The effect on the number of honeybees seeking syrup from 10 dishes of allowing a source of sugar syrup to dry up and then replenishing it with a fresh supply. First day of experiment.

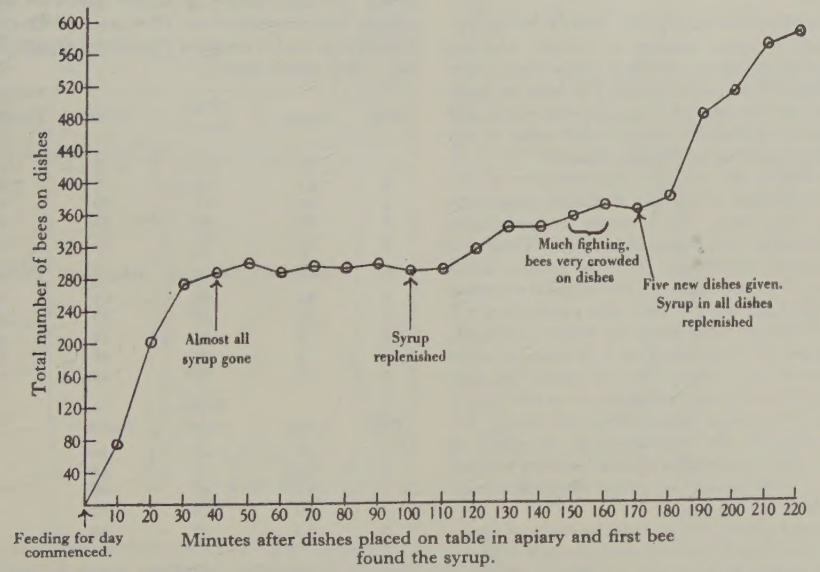

Fig. 2. Arrangement as in Fig. 1 but with higher population of bees since several hundred bees had learned to visit the training table in search of syrup on the previous day. Second day of experiment.

syrup at the table were made at 10 min. intervals. In the first experiment (Fig. 1) almost all the syrup had been taken 110 min. after the first bee had arrived at the table, and counts made showed that between 200 and 220 bees were by this time visiting the table regularly. After a further 60 min. the syrup in all the dishes was replenished and counts continued at 10 min. intervals for a further 50 min. In the second experiment (Fig. 2), carried out on the following day on the same site and in the same manner, a large number of bees, probably those which had learned to seek nectar at this site on the previous day, were again visiting the table regularly in a few minutes. 40 min. after the first bee of the day had commenced to feed the dishes were almost dry, and they were refilled with fresh syrup 1 hr. later. On account of the large number of bees visiting the table by this time (about 350) the dishes were dry once more within 1 hr. and, 10 min. later, all dishes were refilled with syrup and, in addition, a further five dishes of syrup put out. Counts were continued at 10 min. intervals for a further 50 min.

It therefore appears that the quantity of nectar present in the flowers of a particular species of plant is likely to have a great influence on the number of *new* foraging bees which will be attracted to this source of food, but that the quantity of nectar present, always provided that there is some, has little immediate effect upon the number of foragers that have been working the flowers of this species of plant for some time.

Effect of nectar concentration on visits by honeybees

The kinds of sugar present in nectar, whether sucrose or simpler sugars, and their proportions, vary with the different species of plant. The kind of sugar present was not considered in the present work, and all estimations of nectar concentration refer to the percentage of total carbohydrate present.

Table 1 shows the relative concentration of the nectar of a number of species of plants commonly visited by honeybees. Each nectar concentration given in this table is the mean of between fifteen and twenty separate estimations made at various times during 1940, 1941 and 1942, all the samples of nectar being collected between 10 a.m. and 2 p.m. (G.M.T.). Although the above table gives the mean concentrations of nectar as determined on a number of occasions, it should be realized that the limits are very wide and nectars of a concentration of as low as 4% and as high as saturation point sometimes occur. For example, although the concentration of nectar of hawthorn in the Harpenden district seldom exceeds 18%, it was undoubtedly very near saturation point for a few days during 1943.

Tables 2 and 3 show clearly that the range of nectar concentration in the flowers of many species of plants growing in a given district varies greatly from day to day and even from hour to hour. Such changes are almost certainly directly connected with the atmospheric humidity and the presence or absence of dew or rain. The volume and concentration of any drop of nectar varies as the above factors vary on account of the hygroscopic properties of nectar quite apart from any influence which these factors may very well have on nectar secretion itself. In the case of those

Table 1. *Mean concentration of nectar of various flowers arranged in descending order of nectar concentration. (Concentration of nectar expressed as percentage total carbohydrate to nearest whole number.)*

Flower	Mean nectar conc. %
Apple	42
Charlock (*Raphanus Raphanistrum* L.)	40
Savoy cabbage (*Brassica* sp.)	39
Raspberry (*Rubus idaeus* L.)	37
Dandelion (*Taraxacum Dens-leonis* Desf.)	34
Sainfoin (*Onobrychis viciaefolia* Scop.)	34
Wild cherry (*Prunus Cerasus* L.)	33
Lime (*Tilia platyphyllos* Scop.)	31
Wild white clover (*Trifolium repens* L.)	30
Blackberry (*Rubus fruticosus* L.)	28
Bird's-foot trefoil (*Lotus corniculatus* L.)	26
Black currant (*Ribes nigrum* L.)	25
Plum	21
Pear	15
Hawthorn (*Crataegus Oxyacantha* L.)	13

Table 2. *Range of nectar concentrations in the flowers of a number of species of plants from day to day. Nectar was collected on each day between 11 a.m. and 12 noon* G.M.T. *for five consecutive days during the flowering period. (Concentration of nectar expressed as percentage total carbohydrate, to nearest whole number, in each case and determined from two samples on each day. Only mean shown.)*

Day	Apple	Wild cherry	Plum	Dandelion
1	42	21	7	50
2	53	38	9	55
3	50	29	36	24
4	56	52	38	14
5	32	20	34	15
Day	Hawthorn	Charlock	Raspberry	Blackberry
1	22	58	19	29
2	10	47	37	28
3	2	49	39	30
4	3	30	28	31
5	13	25	29	11
Day	Lime	White clover	Sainfoin	
1	46	12	30	
2	49	17	30	
3	55	26	28	
4	50	35	17	
5	33	36	29	

flowers in which the nectaries are relatively unprotected (apple, raspberry, hawthorn, etc. as compared with clovers, dandelion, etc.), dew or rain actually may enter the corolla tube and dilute the contained nectar.

Table 3. *Range of nectar concentrations in the flowers of four species of plants during successive hours of the day.* (*Concentration of nectar expressed as percentage total carbohydrate in each case to the nearest whole number.*)

Plant	Time of day (G.M.T.)										
	A.M.						P.M.				
	7	8	9	10	11	12	1	2	3	4	5
Dandelion	—	25	26	26	26	27	27	27	27	27	27
Hawthorn	—	4	6	11	13	13	13	13	13	13	13
Raspberry	19	20	20	26	35	36	36	37	36	36	36
White clover	21	21	22	24	24	26	27	27	27	27	27

In the case of the flowers the nectar concentrations of which are shown in Tables 2 and 3, the nectar, if collected by hand, was taken from flowers selected at random, that is to say open flowers of all ages. In the many cases when the nectar was taken from the honey-stomachs of bees, there is reason to suppose that this nectar was also collected from flowers of all ages since no evidence was obtained to indicate that the bees were selecting flowers of any particular age when seeking nectar from the species of plants studied. That the figures might otherwise have been seriously biased has been shown by Vansell (1942) who found that in the case of Manzanita (*Arctostaphylos* spp.) those plants most advanced in flowering afforded the richest nectar and were selected by bees in preference to plants of the same species with recently opened flowers.

Fig. 3 clearly demonstrates that honeybees respond to the concentration of nectar secreted by the flowers of plants, working in the greatest numbers on those plants with the highest nectar concentration. The

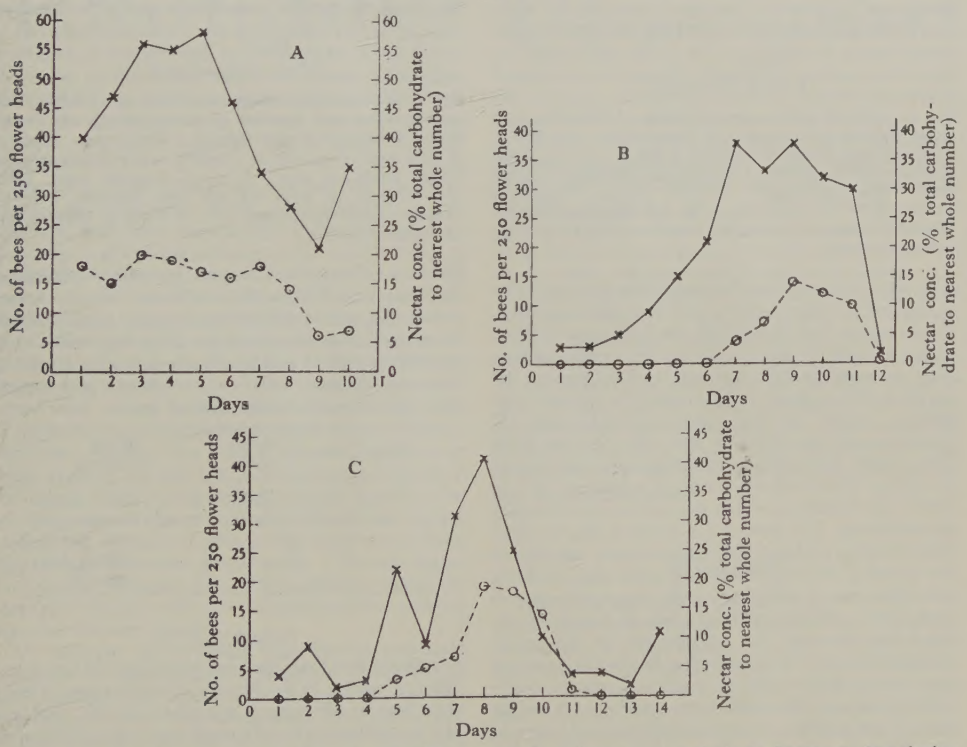

Fig. 3. Variation in the number of honeybees seeking nectar from three species of plants for 10–14 days during their flowering period, and nectar concentration in the flowers during this period. All nectar samples collected and counts of bees made (per 250 flower heads) between 11 a.m. and 12 noon G.M.T. Two nectar samples taken from bees on each occasion and mean concentration given. *A* = apple. *B* = plum. *C* = hawthorn. ×·····× = nectar conc. % (to nearest whole number). ⊙----⊙ = number of bees per 250 flower heads.

nectar samples for Fig. 3 were all collected from the honey-stomachs of bees working on plants growing about 100 yd. from the area where the counts of honeybees were made, since it was necessary to avoid reducing the population of bees in the counting areas artificially. The results shown in Fig. 3 are in close agreement with similar observations made by Vansell (1942) and also with the feeding experiments conducted by Butler *et al.* (1943) and Von Frisch (1938).

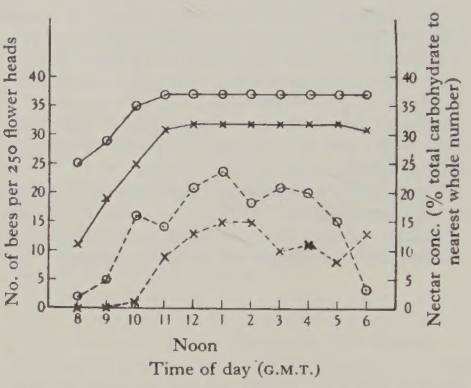

Fig. 4. Relative nectar concentrations of dandelion and apple flowers, and number of bees visiting 250 flower heads of these plants throughout a day in 1940. (Counts of bees made as quickly as possible on previously selected flower heads in sun.) ○——○ = Conc. dandelion nectar. ⊙- - -⊙ = No. of bees on 250 dandelion flowers. ×——× = Conc. apple nectar. ×- - -× = no. of bees on 250 apple flowers.

The latter showed that the higher the concentration of the 'nectar' (sucrose in water) the more vigorously the bees that had found it in the field danced upon their return to the hive, the larger the number of bees they attracted by their dancing, and the greater the number that subsequently visited the dishes containing 'nectar' of the higher concentrations. It therefore appears probable that when two or more 'bee-plants' are in flower simultaneously the species or variety which will attract most honeybees will be that in the flowers of which the nectar is most highly concentrated. It is known, for instance, that in some East Anglian orchards in or near which dandelions are growing, the honeybees and other bees available for purposes of pollination will frequently almost completely neglect the fruit trees in favour of the dandelion flowers. An examination of the nectar concentrations of the competing flowers in one such case at hourly intervals throughout a warm, sunny day in 1940 gave the results shown in Fig. 4. A similar case of competition, between the flowers of pear and hawthorn, is shown in Fig. 5. Vansell (1942) has described another case of competition, a multiple case, between the flowers of apple, peach and nectarine, plum, sour cherry, winter Nelis pear and Bartlett pear in which the mean nectar concentrations were on one day 46·2, 28·9, 25·8, 23·5, 9·9 and 7·9% respectively, with the result that the two varieties of pear were almost completely neglected by the bees present in favour of the apple and other flowers. A similar, but smaller, case of competition between the flowers of a number of kinds of fruit trees growing in the same orchard in Hertfordshire is shown in Table 4 below.

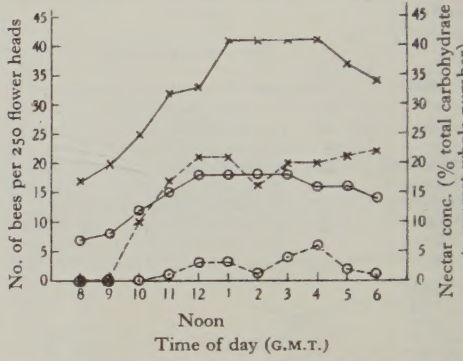

Fig. 5. Relative nectar concentrations of hawthorn and pear flowers, and number of bees visiting 250 flower heads of these plants throughout a day in 1941. (Counts of bees made as quickly as possible on previously selected flower heads in sun.) ○——○ = nectar conc. pear. ⊙- - -⊙ = no. of bees on 250 pear flowers. ×——× = nectar conc. hawthorn. ×- - -× = no. of bees on 250 hawthorn flowers.

Table 4. *Competition between a number of kinds of fruit trees in a Hertfordshire orchard. Nectar concentration (% total carbohydrate to nearest whole number) determined from samples taken from bees collected at random between 11 a.m. and 12 noon* G.M.T. *Counts of bees on fruit trees made between 1 and 2 p.m.* G.M.T. *Four counts made on each kind of tree.*

Pear	Plum	Apple (Cox)	Apple (Grieve)	
11%	16%	32%	37%	Conc. nectar
0·0	0·75	24·75	27·25	No. bees per 250 flowers (mean of 4 counts)

DISCUSSION

It appears that both the concentration and the abundance of nectar in the flowers of various plants has a considerable effect upon honeybee activity, that is to say, upon the number of honeybees visiting the flowers in question in search of nectar at any given time. The two factors 'nectar concentration' and 'abundance' are, however, very closely knit together and their individual influences usually overlap to a considerable

degree, so that in the field it is often by no means easy to determine where the influence of one of these factors ceases and that of the other begins. Very broadly speaking, however, it appears correct to say that nectar concentration decides in the first instance which species of plants will be visited in preference to others in flower at the same time, and that nectar abundance then comes into play in determining whether a greater or lesser number of honeybees will work the flowers in question. Nectar concentration appears to be very largely the species determiner, nectar abundance the population determiner.

Quite apart from the difficulties in assessing the parts played by each of these factors in determining in which direction honeybees' foraging activities will be guided, it is clear that further difficulties will be introduced by the influence of other factors. For example, Butler (1941) was able to show that frequently the factor which determined whether honeybees would or would not visit the flowers of red clover (*Trifolium pratense*) in search of nectar was the accessibility of that nectar to the relatively short-tongued honeybee. On occasion the nectar was found to be more abundant (volumetrically) and of as high, or higher, concentration in the flowers of red clover as it was in the flowers of white clover (*Trifolium repens*) growing in the vicinity, yet no honeybees would be found working the red clover for nectar because the latter was still out of reach of their tongues.

Similarly, there is reason to suppose that the direction of the prevailing wind, particularly in windy districts, may have considerable influence upon the direction in which the honeybees will choose to fly on leaving their hive and, therefore, where they will seek forage and to which of a number of fields they will go. They may thus never find a field containing flowers with more favourable nectar qualities than those of the flowers in their preferred line of flight. There is little doubt that this factor of wind direction, combined with colony location relative to the positions of the flowering crops available at the time and within the flight range of the bees concerned, accounts in large measure for the frequently observed fact that the honey yield in one apiary may be one-third as much again per colony compared with another apiary containing colonies of bees of the same race, strain and strength, situated a mile away. This may occur even though the same crop of flowers from which the most and richest nectar is to be gathered lies midway between the two apiaries.

Yet another factor which may have a profound influence in determining to which of a number of species of plants in flower at the same time honeybees will resort in search of nectar is the commencement of the flowering periods of the species concerned, together with the rate of increase of the foraging populations of the colonies of bees in the neighbourhood. If a species of bee plant commences to flower before another species, it will, other things being equal, attract a large population of foraging bees. Many of these will, as shown by Butler *et al.* (1943), become 'fixed' bees and will not migrate on to the second species of plant when its flowers open, even though the latter may contain a more abundant and more concentrated nectar, provided that the first crop is still in flower. The bees that will find their way to the newly available flowering crop will be 'wandering' bees which have not yet become 'fixed' to any particular foraging site on the earlier crop. These will be mostly young bees foraging for the first time, and the rate of increase of the foraging population on this second crop will be proportional to the rate of increase in the number of new foraging bees in the colonies of bees in the neighbourhood so long as no extraneous factors, such as dying off of the first crop or competition from yet another plant species, are introduced to complicate matters further.

No study has yet been made of the effect of nectar composition on honeybee activity. It is known that considerable variations occur in the percentages of the different sugars contained in nectars of different plant species, and it appears reasonable to suppose that such differences in the contained sugars may help to determine which of a number of plant species honeybees will prefer. Similarly, so far as is known, no work has yet been published on the factors which determine from which plant species honeybees will seek pollen. The choice of pollen plants by the honeybee may influence its choice of nectar plants to some extent, since it is believed that a proportion of the foraging population of a colony of honeybees are deliberate pollen-nectar gatherers, not merely gathering pollen incidentally (accidentally or involuntarily) on the same trip upon which they seek nectar.

It seems clear that there are many factors, which frequently interact with or against one another, influencing the choice of plant species which a honeybee will work at any given time and in any given district, and it is rather surprising that a study of only two of these factors, nectar abundance and nectar concentration, should in many cases apparently lead to the possibility of drawing fairly definite conclusions as to why one species of bee plant is deserted and another favoured.

SUMMARY

1. As the quantity of nectar of a given species of plant becomes smaller, irrespective of its sugar concentration, so the number of bees working that species of plant becomes reduced. This is not due to old bees that have been working this crop for some time deserting it in favour of a more profitable crop, but to the natural death of these old bees and the fact that their places are not taken by new bees. Young foragers are not attracted to the crop concerned because its presence is not communicated to them by the old bees still working upon it.

2. The range of nectar concentration (% total carbohydrate) in the flowers of many species of plants growing in a given district varies from day to day and even from hour to hour. Such changes are almost certainly directly due to changes in the atmospheric humidity. The changes are greatest in flowers, such as hawthorn, with relatively unprotected nectaries.

3. 'Honeybees respond to the concentration of nectar secreted by the flowers of plants, working in the greatest numbers on those plants with the highest nectar concentration.

4. When two or more species of 'bee-plants' are in flower simultaneously the species which will attract most honeybees will be that in the flowers of which the nectar is most highly concentrated. This accounts for the occasional failure of honeybees to work the flowers of fruit trees when other flowers are available to them.

5. Both nectar abundance and nectar concentration appear to have considerable effect upon honeybee activity. From the data at present available it appears correct to conclude tentatively that nectar concentration decides in the first instance which species of plant will be visited in preference to others in flower at the same time, and that nectar abundance then determines the proportion of the foraging population of a colony which will work the flowers in question.

I wish to take this opportunity of thanking Dr R. K. Schofield for the loan of the Lovibond Tintometer used in these investigations and his interest in the work.

REFERENCES

BUTLER, C. G. (1941). *Ann. Appl. Biol.* **28**, 125–34.
BUTLER, C. G. & FINNEY, D. J. (1942). *J. Exp. Biol.* **18**, 206–12.
BUTLER, C. G., JEFFREE, E. P. & KALMUS, H. (1943). *J. Exp. Biol.* **20**, 65–73.
FRISCH, K. VON (1938). *Smithson. Rep.* (Publication 3511), pp. 423–31.
PARK, O. W. (1933). *Iowa St. Apiarist Rep.* pp. 22–9.
PIRIE, N. W. (1936). *Brit. J. Exp. Path.* **17**, 269–78.
VANSELL, G. H. (1942). *Circ. U.S. Dep. Agric.* no. 650.

THE DISTRIBUTION OF VITAMIN C IN *NYCTOTHERUS CORDIFORMIS* EHRENBERG, *OPISTHIOGLYPHE RANAE* FRÖLICH, AND *TOXOCARA CANIS* WERNER

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(With Five Text-figures)

INTRODUCTION

The possibility that internal parasites may play a part in the vitamin metabolism of the host was first suggested by Roskin & Nastiukova (1941). Working on *Trypanosoma brucei* in guinea-pigs, these workers demonstrated the presence of vitamin C granules in the cytoplasm of the parasite. They further showed that the trypanosomes are capable of absorption of the vitamin, since an increase in the ascorbic acid content of the blood of the host, brought about by injection, was rapidly followed by a corresponding increase in the quantity of the vitamin C in the parasites as demonstrated by staining with the specific vitamin C reagent.

The possibility that a similar absorption may take place in larger parasites has led us to investigate the localization of vitamin C in the ciliate *Nyctotherus cordiformis* Ehrenberg, the nematode *Toxocara canis* Werner (syn. *Belascaris marginata*), and in the trematode *Opisthioglyphe ranae* Frölich.

MATERIAL AND METHODS

The nematodes were obtained from a post-mortem examination of the alimentary canal of a dog which had previously been fed on a normal mixed diet. The ciliates and trematodes were obtained from the alimentary canal of a freshly killed frog. The organisms, after removal from the host, were rinsed in saline to remove the intestinal debris, washed quickly in distilled water and fixed as described below. The nematodes were cut into small sections before fixation.

The basis of the histological demonstration of vitamin C is that under certain conditions, only this substance will bring about the reduction of silver nitrate to metallic silver. The technique was first introduced by Szent-Györgyi (1928), but the difficulty of introducing a fixing agent into the reagent was not solved until Giroud & Leblond (1934) produced a very specific reagent by using acetic acid combined with silver nitrate. Bourne (1936) has modified this reagent somewhat and it is his technique that is used in the present paper. This method is as follows:

The reagent consists of 5 % silver nitrate combined with a 5 % solution of acetic acid in the proportion of 5 c.c. of acid to each 100 c.c. of the silver solution. It is important to use the purest silver nitrate obtainable; B.D.H. 'Analar' salts were used in the present research. In the preparation and use of the reagent, absolute cleanliness in the preparation of the glassware is essential. All containers were first washed in soap and water, secondly with chromic acid, and finally rinsed with tap water, followed by glass-distilled water. Impregnation of the tissues was carried out in the dark to prevent possible decomposition of the silver salt by the action of light. The most satisfactory period was found to be about 20 hr. for the helminths, and 2–3 hr. for the ciliates. After impregnation, the unreduced silver was removed by placing the tissues in photographic 'hypo' for 2 hr. This was followed by several changes of distilled water—about an hour in each. The helminths were dehydrated, cleared, and embedded; sections were cut at 5μ. The ciliates were concentrated by centrifuging, and mounted whole by the albumen film method (Smyth, 1944).

Preparations were counterstained in 1 % orange G in absolute alcohol; toning in gold chloride was also used to intensify the reduced silver in the tissues. A few preparations were treated with 5 % ammonia solution following a suggestion by Bourne, Barnett & Fisher (1941) that this increases the specificity of the method in some cases. It was found that with the present organisms, this treatment in no way altered the cytological picture as compared with that obtained in untreated preparations.

VALIDITY OF THE VITAMIN C REACTION

The fact that vitamin C is the only substance that will reduce silver nitrate in the presence of acetic acid has been proved by many workers, and it is not proposed to discuss the question here in any detail. Harris (1933) has shown that the tissues which give the highest figures for vitamin C content by titration also stain most intensively with silver

nitrate; he further demonstrated that in progressive scurvy, the decrease in ability of the tissues to reduce silver nitrate is compatible with the decrease in amount of vitamin C as estimated by titration. The silver nitrate-acetic acid reagent has been used by numerous workers especially on vertebrate glandular tissue, and there seems little doubt as to its specificity. Recently, some objections have been put forward by Barnett & Fisher (1943) from the results of staining mixtures of gelatin and ascorbic acid, and ground glass and ascorbic acid, but according to Bourne (1944) it is doubtful whether their results have any true significance.

OBSERVATIONS

Nyctotherus. Preparations of this ciliate showed that the vitamin C was distributed uniformly throughout the endoplasm in the form of spherules or disks of varying size (*V*, Fig. 1). These were never found in the ectoplasmic layer (*E*, Fig. 1) of the cytoplasm, nor were any present in the region of the mega- or micronucleus. In contrast with the uniform distribution of vitamin C in *Nyctotherus*, it is interesting to note that no trace of vitamin granules of any type were found in the cytoplasm of *Opalina* obtained from the same frog.

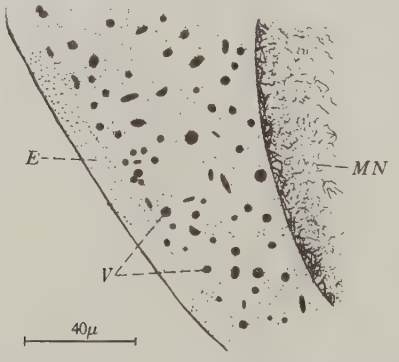

Fig. 1. Portion of *Nyctotherus* stained by the silver nitrate-acetic acid technique. Camera lucida drawing. *E*, ectoplasm; *MN*, meganucleus; *V*, vitamin C granules.

Toxocara. A brief note on the localization of vitamin C in this organism has already been published (Smyth & Hill, 1944). Fig. 2 shows a transverse section of *Toxocara*, and it can be seen that the vitamin is concentrated in considerable quantity in the cells of the gut. These cells, shown enlarged in Fig. 3, are laden with heavily impregnated granules (*V*) situated in the region between the nucleus and the free cell border, the densest region of impregnation being that just above the nucleus. A few granules are found just beneath the wall of the free border and a series of fine granules are present below the nucleus and lining the lower cell wall where it meets the external cuticle covering the gut (*B*, Fig. 3).

Compared with the intestinal cells, the amount of vitamin distributed in the remaining tissues is exceedingly small. A few granules are found in each of the cells of the reproductive tubules (*R*, Fig. 2), but these in contrast with the granules of the gut cells are small and only visible under oil immersion. In the longitudinal muscles (*M*, Fig. 2) very fine diffuse grains are present. In all the other tissues—nerve cells, epidermis, etc.—only very few scattered grains are visible. In the section shown in Fig. 2, an elongated blackened bleb (*K*) can be seen in the body cavity. Since the food (*F*, Fig. 2) in the gut lumen shows some vitamin C present, it seems possible that this bleb represents a particle of food material which found its way into the body cavity when the nematode was cut into slices before fixation.

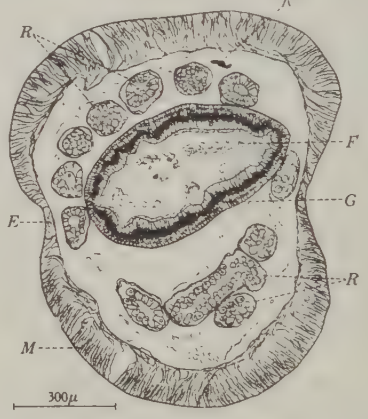

Fig. 2. Transverse section of *Toxocara* with external cuticle removed. Camera lucida drawing. Silver nitrate-acetic acid preparation. *E*, excretory canal; *F*, food in gut; *G*, gut cells laden with vitamin C granules; *K*, artifact; *M*, longitudinal muscles; *R*, reproductive tubes.

Opisthioglyphe. In this trematode, in contrast with *Toxocara*, the cells of the gut are completely lacking in granules of vitamin C. The distribution is largely confined to the thin walls of the excretory vessels. In *Opisthioglyphe*, the excretory system takes the form of two longitudinal canals which join to form a single median vessel in the posterior third of the body before passing to the posterior excretory pore. In Fig. 4 the section has been taken at the point of junction of the lateral excretory vessels. The walls of each of these is lined with impregnated granules of vitamin C, and the transverse connecting region (*I*, Fig. 4) shows an even heavier impregnation. In Fig. 5 the excretory canal is single but widened considerably preparatory to dividing into two. Here, too, the same impregnation is observed.

The vitamin is also seen in all sections as a very thin line of flattened granules lying beneath the epidermis (*L*, Fig. 4, 5). Throughout the remainder of the tissues very little vitamin is present. A few scattered granules are present in the parenchyma, longitudinal and diagonal muscle layers, and a small quantity is also localized in the tissues of the testes and the yolk glands.

Fig. 3. Intestinal cells of *Toxocara*. Silver nitrate-acetic acid preparation. Camera lucida drawing. *B*, cuticular covering of gut; *N*, nucleus; *V*, vitamin C granules.

DISCUSSION

The problem which now presents itself is how the presence of the vitamin in the tissues is to be interpreted. Since the parasites obtain their nourishment from the host, the most reasonable conclusion to be drawn is that they are able to absorb the vitamin from the food or tissues of the host. Indeed, if the host is fed on a normal mixed diet, we should expect such an absorption to take place. It is difficult to account for the absence of any vitamin granules in the astomatous *Opalina*, as compared with the presence of numerous granules in the cytoplasm of the stomatous *Nyctotherus*. The fact that this latter ciliate is a detritus feeder, whereas *Opalina* takes in nourishment over its whole body surface, may account for this difference.

In *Toxocara*, the fact that such relatively large concentrations of vitamin C are found in the gut cells is very strong evidence that absorption of vitamin takes place from the food of the host. Hirsch (1939) has shown that if isolated strips of intestine from a starved *Ascaris* are incubated in a 0·1 % solution of vitamin C, the intestinal cells absorb the vitamin which becomes concentrated in the Golgi apparatus. In *Toxocara*, the presence of granules below the free border of the gut cells possibly represents the process of absorption actually taking place. It is interesting to note that in the turbellarian

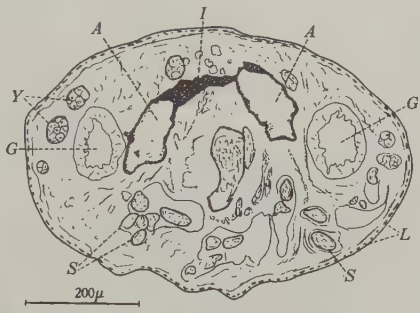

Fig. 4. Transverse section of *Opisthioglyphe* showing junction of lateral excretory canals. Silver nitrate-acetic acid preparation. *A*, lateral excretory vessels; *G*, gut; *I*, junction of lateral canals heavily laden with vitamin C; *L*, layer of vitamin C grains beneath epidermis; *S*, eggs in uterus; *Y*, yolk glands.

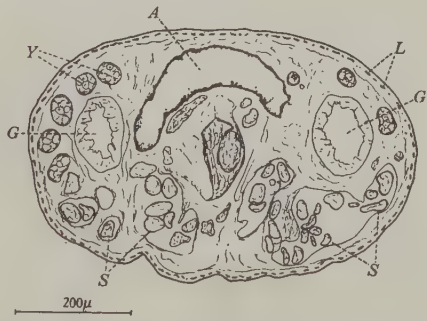

Fig. 5. Transverse section of *Opisthioglyphe* showing median excretory vessel prior to division. Camera lucida drawing. Silver nitrate-acetic acid preparation. *A*, median excretory vessel laden with vitamin C granules; *G*, gut; *L*, layer of vitamin C granules beneath epidermis; *S*, eggs; *Y*, yolk glands.

Dendrocoelum lacteum, a similar localization of the vitamin in the gut has been demonstrated by one of us (G. R. H., unpublished). Moreover, in specimens of *Dendrocoelum* placed in a weak solution of vitamin C (0·005 %), the number and size of the granules became increased appreciably, indicating that absorption of vitamin C via the gut cells could readily take place.

The results obtained in *Opisthioglyphe* are surprising in view of the distribution of the vitamin in

Toxocara, and it is difficult to put forward any reasonable hypothesis to account for the almost exclusive localization of the vitamin to the excretory system. It was thought that the peculiar distribution might possibly be due to faulty technique or impure reagents, but repetition of the entire experiment using freshly prepared reagents and further specimens of *Opisthioglyphe* from different frogs, yielded identical results. It is difficult to see how the vitamin comes to be localized in the excretory canals if it is not absorbed by the cells of the gut in the first instance. Until further experimental work is carried out on this organism no definite conclusion can be reached.

The fact that parasites may be capable of absorption of vitamin C from the food or tissues of the host, may well lead to a revision of our theories of the host-parasite relationship. Many of the effects of parasites are due to the absorption of food material, especially in the case of nematodes. The great concentration of vitamin C in the gut cells of *Toxocara* seems to justify the conclusion that absorption of the vitamin has taken place. If a similar absorption takes place in human nematodes, as Hirsch's results with isolated intestinal tissue of *Ascaris* seem to indicate, it is possible that in cases of very heavy infection the whole vitamin C metabolism will be thrown out of order. It is doubtful whether in a well-nourished individual sufficient quantities of the vitamin could be absorbed to produce harmful results. In an under-nourished organism, however, the vitamin C content may just be sufficient to prevent scorbutic conditions, and the introduction of nematodes in quantity may have the effect of sufficiently upsetting the vitamin C balance to induce scorbutic conditions.

As regards the ciliate studied, the same conclusions arrived at for the nematode may also be deduced. It is very doubtful whether intestinal ciliates are ever present in sufficient numbers to absorb an appreciable quantity of the vitamin. It is possible, of course, in the case of blood Protozoa—as was pointed out by Roskin & Nastiukova (1941)—that organisms such as trypanosomes may be present in sufficient quantity to produce an appreciable absorption.

Further experimental work will be needed, especially on the effect of parasites on the metabolism of normal and scorbutic animals, before any generalized conclusion as to the part played by parasites in the vitamin C equilibrium of the host can be forthcoming. But it seems advisable that in considering the pathological effects of parasites—and especially nematodes—the possibility of vitamin absorption should not be overlooked.

SUMMARY

1. The localization of vitamin C in *Nyctotherus cordiformis*, *Toxocara canis* and *Opisthioglyphe ranae* was investigated by means of the silver nitrate-acetic acid technique.

2. In *Nyctotherus*, the vitamin C is distributed uniformly throughout the cytoplasm in the form of globules of varying sizes. No vitamin is present in the ectoplasmic layer or in the region of the nuclei. In the same preparations specimens of *Opalina* show no trace of any vitamin C present.

3. In *Toxocara*, the vitamin is localized in relatively large quantities in all the cells of the intestine. In these cells it is aggregated mainly in the region between the nucleus and the free cell border. A series of fine granules of the vitamin also line the lower cell walls. Only a few scattered granules are present in the remaining tissues.

4. In *Opisthioglyphe*, the vitamin is concentrated on the walls of the excretory system which in this trematode has the form of two longitudinal canals joining in the posterior region to form a single median canal. A line of disk-like elements of the vitamin is also present immediately below the epidermis. In the remaining tissues only very small scattered grains are found.

5. It is believed that in the case of *Toxocara*, the presence of the vitamin in the gut cells may indicate that the parasite is capable of absorption of vitamin C from the food of the host.

6. It is suggested that in heavy parasitic infections the parasites may play some part in the disturbance of the vitamin C balance of the host.

REFERENCES

BARNETT, S. A. & FISHER, R. B. (1943). *J. Exp. Biol.* **20**, 14.
BOURNE, G. H. (1936). *Anat. Rec.* **66**, 3.
BOURNE, G. H. (1944). *Nature, Lond.*, **153**, 254.
BOURNE, G. H., BARNETT, S. A. & FISHER, R. B. (1941). *Nature, Lond.*, **147**, 542.
GIROUD, A. & LEBLOND, C. P. (1934). *C.R. Soc. Biol., Paris*, **115**, 705.
HARRIS, G. C. (1933). *Nature, Lond.*, **132**, 27.
HIRSCH, G. C. (1939). *Form und Stoffwechsel der Golgi-Körper*. Protoplasma Monographs. Berlin.
ROSKIN, G. & NASTIUKOVA, O. (1941). *C.R. Acad. Sci. U.R.S.S.* **33**, 8 (abstract).
SMYTH, J. D. (1944). *Science* (in the Press).
SMYTH, J. D. & HILL, G. R. (1944). *Nature, Lond.*, **153**, 21.
SZENT-GYÖRGYI, A. (1928). *Biochem. J.* **22**, 1387.

ON THE BEHAVIOUR OF WIREWORMS OF THE GENUS *AGRIOTES* ESCH. (COLEOPTERA, ELATERIDAE) IN RELATION TO TEMPERATURE

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(With Thirteen Text-figures)

INTRODUCTION

The work described in this paper represents an attempt to obtain as complete a picture as possible of the influence of temperature on the behaviour of wireworms, in so far as behaviour affects their activity and 'choice' of habitat. For this purpose it was necessary to pursue a number of more or less independent lines of investigation, the results of which are for convenience presented together here.

Since the behaviour of wireworms is subject to the influence of environmental factors other than temperature—humidity and moisture in particular (Lees, 1943 *a*, *b*)—it is not thought desirable to attempt to relate the results of this laboratory study of one isolated factor to the activities of wireworms under natural conditions, such as are concerned with the periodicity of the damage to crops and the supposed seasonal migrations. For this reason the rather scanty information which is available about the temperature conditions in the soil will not be reviewed here, except in so far as it bears on particular points.

For the purpose of analysis it is convenient to distinguish two ways in which temperature may influence the behaviour of an invertebrate. In the first place the animal's activity may vary in amount according to the temperature; and secondly, the animal may orientate itself with respect to differences of temperature in the environment in such a way that it avoids temperatures above or below certain limits, and thus exhibits a 'preference' for a particular range of temperature. In the analysis of the behaviour of wireworms in response to temperature, which is described here, these two effects of temperature have been investigated separately, the effect on activity being described in Section II, and that on orientation in Section III.

It was necessary for this study to carry out some preliminary experiments in order to determine approximately the range of temperature over which wireworms can maintain life, since the previous work on the upper and lower thermal death-points of wireworms was inadequate. It was thought that the information obtained would be of ecological interest, for by examination of the published records of soil temperature it would be possible to show whether temperatures lethal to wireworms are ever likely to occur at any particular depth in the soil, and thus to decide whether extremes of temperature are to be considered as a possible factor of importance in controlling the vertical distribution of wireworms. This work is described in Section I.

MATERIAL

The material was obtained from fields in Cambridgeshire and consisted of the larvae of three species of *Agriotes*. Of these, *A. sputator* was distinguished from the other two by means of the characters described by Guéniat (1937), and was used in some of the experiments in Section I. This species was, however, not always abundant, and most of the work was done with mixed material consisting of *A. lineatus* and *A. obscurus* in unknown proportions, these two species being indistinguishable in the larval stages. This *A. lineatus-obscurus* material was used exclusively in the work on resistance to low temperatures and in the experiments on behaviour (Sections II and III), since much of the previous physiological work on wireworms has been done with this mixed material.

Large larvae were used throughout, those of *A. sputator* being from 10 to 17 mm. in length, and those of *A. lineatus-obscurus* from 13 to 24 mm.

I. LETHAL TEMPERATURES

No previous work dealing with the upper thermal death-point of *Agriotes* was found in the literature, though there is a small amount of information concerning a related genus, *Melanothus*, to which reference will be made in the discussion. The resistance of wireworms to low temperatures has been investigated by two previous workers, but the published

* The work was carried out during the tenure of a Studentship from the Carnegie Trust for the Universities of Scotland.

results are few and indefinite. Langenbuch (1932) found that old *Agriotes* larvae taken from the field in winter could survive exposure 'for several hours' to a temperature of −14°C. Guéniat (1937) left five *Agriotes* larvae in a metal box on the surface of the soil in Switzerland from mid-December to April. During this period the air temperature (and presumably also the temperature in the box) reached −10°C. several times, and −15°C. once. All five wireworms survived. The same author found, from his sampling, no evidence of a winter mortality, nor of a downward migration to warmer layers during the winter. This previous work suggests that wireworms can resist very low temperatures, such as would not be expected to occur in England, and the work described here was designed to show whether this was the case for wireworms obtained in England.

Resistance to high temperature

Technique

The object of the work was to find the highest temperature which could be tolerated by the wireworms for an indefinite period. This was done by observing the length of time for which the animals were able to survive when exposed to various lethal high temperatures, and estimating from these observations, by extrapolation, the highest temperature at which they would be expected to survive indefinitely. The larvae were observed individually and the time at which each became paralysed was noted. This was taken as the time of survival. A preliminary experiment showed that the exposure required to paralyse was considerably shorter than that required to kill the animals, but the highest temperature which could be withstood indefinitely was the same (within 1°C.) in each case.

The observations were made on batches of five wireworms in a glass jar immersed in a water-bath which was heated electrically and controlled by a thermostat. The cork of the jar carried a thermometer with the bulb in contact with the bottom of the jar which was covered with damp filter paper. When the jar had attained the desired temperature the wireworms were introduced through a small hole in the cork, which was then closed by a glass rod. The wireworms were watched continuously and the time at which each of the five became paralysed was noted. The onset of paralysis proved to be difficult to judge; the animals' movements became gradually less under the influence of high temperature, and there was no definite point at which movement ceased. The criterion of paralysis adopted was that the animal should remain still for 1 min. or more.

In specifying a lethal temperature it is necessary to state not only the temperature and the exposure but also the percentage of animals which are killed (or paralysed) by these conditions. Current practice is unfortunately not uniform in this matter, and various percentages of mortality have been used in specifying the lethal conditions. The fact that there is a certain range of temperature or of exposure within which some, but not all, of the animals will be killed arises from the natural variation among individuals of a species: individuals vary in susceptibility to abnormal temperatures in the same sort of way as they vary in other characteristics. It appears most natural, therefore, to specify, as with other biological variates, the mean value of the susceptibility of the individuals tested: to specify, that is to say, the conditions of temperature and exposure which are just sufficient to kill 'the average individual', or in other words, to produce 50% mortality. The use of 50% mortality has the further advantage that it gives a mean value, the accuracy of which can be easily estimated from its standard error, and comparisons can thus be made between the results of different experiments.

Statistical methods for calculating the mean susceptibility of the animals have been devised in connexion with toxicological work (Bliss, 1935, 1937) and these methods have been applied to the experiments described here, high temperature being regarded as a toxic agent and treated in the same way. The procedure was as follows. By graphical means (Bliss, 1937) it was found that the individual variations in susceptibility to high temperature formed a normal curve of error when the susceptibility was expressed as the reciprocal of the time of survival. To calculate the mean susceptibility, the time of survival of each animal was therefore converted to its reciprocal, and the arithmetic mean of these values and its standard error were then calculated.

As a result of the application of these methods, values were obtained which expressed the mean susceptibility of the wireworms to each experimental high temperature or, in other words, the reciprocal of the time of exposure to each temperature required to produce paralysis in 50% of the sample. By extrapolation from these values it was possible to estimate approximately the temperature at which susceptibility was zero, or the highest temperature which would be tolerated indefinitely by the 'average wireworm'.

The material was obtained in July, and the experiments were performed in August, the animals having been kept previously for at least 2 days at a constant temperature of 16°C. No animal was used in more than one experiment.

Results

1. *Exposure required to paralyse* A. sputator *at different temperatures.* Observations of the time required to induce paralysis in five individual wireworms were made at each of six experimental temperatures from 36 to 43°C. The observations were treated as time-mortality data (Bliss, 1937), and the mean susceptibility of the animals at each temperature was determined. The results are given in Fig. 1. As explained above, the susceptibility is expressed

as the reciprocal of the time of exposure, and is to be read from the left-hand scale. The right-hand scale indicates the time of survival. Extrapolation of the curve shows the susceptibility to be zero at a temperature of between 35 and 36°C. This therefore is the highest temperature at which the wireworms could maintain activity.

2. *Effect of previous temperature.* Experiments were carried out in order to determine the extent to which the previous temperature influenced the resistance of the wireworms to high temperatures. The effects of different periods of exposure to a temperature of 25°C. on the resistance of *A. sputator* to

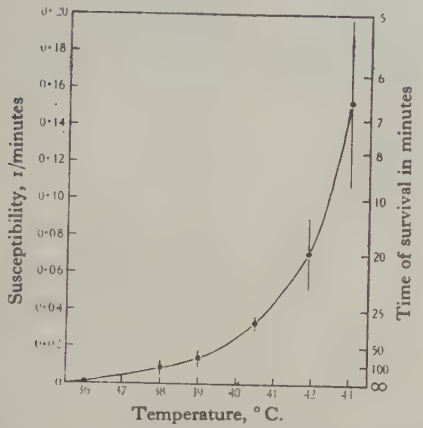

Fig. 1. Lethal high temperature of wireworms. Exposure required to paralyse 50% of sample at various temperatures. Each point is the mean of five observations, and the vertical lines extend to ± twice the standard error of the mean.

paralysis at 39°C. were tested. The animals were kept for 6 days at 16°C. and then divided into four batches of ten. The resistance of the first batch to paralysis at 39°C. was tested immediately, and the others were kept at 25°C. for 12, 24 and 48 hr. respectively before being tested. The effects of different previous temperatures were then tested by determining the resistance of samples of ten individuals of *A. lineatus-obscurus* to paralysis at 39 and at 40°C. after 2 days' previous exposure to temperatures of 6, 16, 25 and 35°C. In both experiments the differences in resistance of the batches were found to be slight and irregular, and were not statistically significant. It is concluded, therefore, that the resistance of the wireworms to high temperatures was not materially influenced by the previous temperature.

Resistance to low temperature

Technique

In the work on the resistance to low temperature, relatively simple methods were used. The animals were put into 3 in. × 1 in. glass tubes containing a soil, obtained by mixing sand and 'fen' soil, which was of loose texture and damp, but with little free water in the pore spaces. These tubes, each containing five or ten wireworms, were subjected in various ways to low temperatures and were removed singly after different periods of exposure. The tubes were then warmed at room temperature (about 15°C.), and after 1 day the wireworms were examined, and the percentage mortality counted. The criterion of death adopted was that the wireworm should not move on being stimulated with forceps.

Four sets of experiments were carried out, of which the first three were done in February with larvae obtained in that month, and the last in the following December with larvae obtained at the end of October. The wireworms were kept in moist sand (20% of saturation) at a constant temperature of 11°C. for a week or more before the experiments.

Results

1. *Rapid freezing: no acclimatization.* The tubes containing the wireworms were transferred directly from a temperature of 11°C. to the experimental low temperature, and the fall of temperature was thus very rapid. Tubes were exposed for different lengths of time to temperatures of 0, −3, −7 and −10°C. (obtained by the use of rooms in the Low Temperature Research Station, Cambridge). No mortality resulted from an exposure of 14 days at 0°C. The mortality after different periods of exposure at −3°C. is shown in Table 1, under treatment 1. Half the animals were found to be dead after 3 days' exposure, and all after 6 days'. At −7°C. all the animals survived an exposure of 1 hr., but all were killed by an exposure of only 4 hr.

This experiment suggested that the wireworms were far from being as resistant to low temperature as the previous work suggested. An experiment designed to test the effect of acclimatization was therefore carried out.

2. *Rapid freezing: acclimatization at* 0°C. The procedure was the same as in the above experiment, but the tubes were kept for 24 hr. at 0°C. before being transferred to the experimental low temperature of −3°C. The mortality after different periods of exposure is shown in Table 1, under treatment 2. It is clear that the resistance of the larvae was not increased by this treatment.

3. *Slow freezing.* Since in the above experiments the fall of temperature had been much more rapid than would probably occur under natural conditions, a further experiment, in which the rate of freezing was somewhat reduced, was carried out. The tubes containing the wireworms were insulated by placing them in a small hay-box. This, with the wireworms in it, was left for 2 days at 0° and then transferred to −3°C. Measurement of the rate of cooling showed that about 5 hr. were required for the tubes to fall

from 0 to $-3°$C. The mortality in the different tubes is shown in Table 1, under treatment 3. All the animals were killed by an exposure of 7 days, and the results do not suggest that their resistance was substantially increased by the slight reduction of the rate of freezing. The fall of temperature was still, however, more rapid than would be expected to occur in the soil, and the following experiment, in which the fall of temperature was controlled, was therefore carried out.

4. *Controlled lowering of temperature.* The experimental temperature was controlled by the use of a refrigerator, and was reduced from $+3$ to $-10°$C. in steps of 1° or 2° per day, as shown in Fig. 2.

The refrigerator was available for only a limited period, and the experiment had to be done without previous testing of the controls, with the result that the lowering of the temperature was not quite regular. There was, moreover, a certain amount of fluctuation

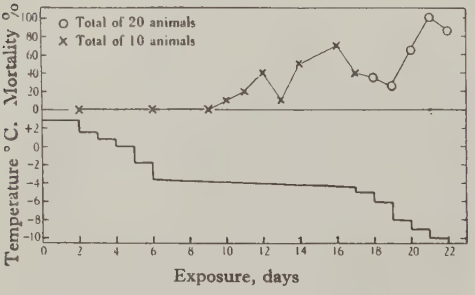

Fig. 2. Record of experiment with controlled rate of freezing. Lower line—temperature graph. Upper line —mortality in samples removed at various stages of the experiment.

of the temperature which could not be eliminated. At the highest temperatures used the range of fluctuation amounted to about 2°C.; at $-4°$ it was 0·5°C., while at $-6°$ it was scarcely visible on the thermograph record. The period of oscillation was about 4 hr. at the highest temperatures and 2 hr. at the lowest.

Twenty tubes each containing ten wireworms were put into the refrigerator at $+2·8°$C. The temperature was then lowered in stages and the tubes removed singly after different periods of exposure, the last being removed after an exposure of 1 day to $-10°$C. When the temperature had reached $-4°$C. it was maintained at that level for some days, so that the tubes removed during this period made possible a comparison of the resistance of the wireworms after the temperature had been slowly lowered to this level with that previously found when the temperature had fallen rapidly to $-3°$C. The mortality found in each tube is shown in Fig. 2. No sample showed a mortality of 100% till a temperature of $-9°$C. had been reached, and even at the end of the experiment, after 1 day at $-10°$C., a few animals survived. During the period of 11 days when the temperature was held nearly constant at about $-4°$C., a mortality of 50% was not produced till after 8 days, and no sample showed a higher mortality than 70% (see Table 1, treatment 4); whereas in the experiments in which freezing was rapid all the wireworms were killed after 6 days at $-3°$C.

It is evident, therefore, that when the temperature was lowered slowly, as in this experiment, the resistance of the wireworms was considerably increased.

Table 1. *Comparison of the results of different treatments on the mortality of wireworms, after exposure to temperatures of* $-3°$C. (*treatments* 1, 2 *and* 3) *or* $-4°$C. (*treatment* 4). *The figures give the percentage mortality, based on the number of animals shown by the figure in brackets.*

Duration of exposure in days	Treatment*			
	1	2	3	4
1	0 (5)	—	—	—
2	33 (15)	40 (5)	0 (5)	—
3	50 (10)	60 (5)	20 (5)	0 (10)
4	90 (10)	—	—	10 (10)
5	80 (10)	100 (5)	60 (5)	20 (10)
6	100 (10)	—	—	40 (10)
7	100 (15)	—	100 (5)	10 (10)
8	—	—	—	50 (10)
9	—	—	—	—
10	—	—	—	70 (10)
11	—	—	—	40 (10)

* *Treatments.*

1. Rapid freezing at $-3°$C. No previous exposure to 0°C.
2. Rapid freezing at $-3°$C. 24 hr. previous exposure to 0°C.
3. Fall from 0 to $-3°$C. in 5 hr. 2 days' previous exposure to 0°C.
4. Fall of temperature in stages of about 2° to about $-4°$C.

It is noteworthy that the increased resistance which was evident after a gradual lowering of the temperature must have been produced by acclimatization at temperatures below 0°C. This is interesting in view of the fact that Mellanby (1939) found that the resistance of the insects with which he worked was not increased by acclimatization at temperatures at which the insects were in chill-coma, and there is no doubt that the wireworms were in chill-coma at 0°C.

Soil temperature

If the information obtained from these experiments is to be related to the ecology of the wireworms, some knowledge of the maximum and minimum temperatures which normally occur in the soil in England is needed. Relevant data are scanty, par-

ticularly for the upper layers of the soil, and it is not possible to state precisely the extreme temperatures to which wireworms are liable to be subjected.

Observations made at Long Ashton, Somerset (Tutin, 1928) showed that at a depth of 4 in., the mean maximum for July 1928 was 28° C., but 29·5° C. was reached on one occasion. Data obtained in 1914 at Rothamsted, Hertfordshire (Keen & Russell, 1921), from a recording thermometer showed that at a depth of 6 in. below bare soil the daily maximum temperature in summer was usually about 22° C., though it rose on one occasion to 26·5° C. Nearer the surface the temperature is, of course, higher, and Russell (1937) quotes a record of 35° C. at ½ in. depth below bare soil on a hot sunny day when the air temperature was 30° C., while Keen & Russell (1921) state that 'the surface of the soil rises to a temperature considerably above that of the air'. This being the case, a more extended view may be obtained than was possible from the above records which covered periods of only 1 year, by referring to the meteorological records. Records from the Radcliffe Observatory in Oxford show that from 1881 to 1930 the absolute maximum air temperature for July (the hottest month) had a mean value of 27·5° C. and the highest value recorded (in August 1911) was 35° C. It seems probable, therefore, that on rare occasions the temperature of the surface layer of the soil exceeds 36° C., a temperature lethal to wireworms.

Concerning the minimum soil temperatures, published records make it clear that the degree of frost which could be experienced by wireworms in England is very slight. Thus, Mellish (1899), summarizing his data, says: 'The Table shows that down to a depth of 1 foot the ground is liable to be frozen occasionally in most parts of England, but that at this depth it is only at a few places that readings below −1° C. are to be expected.' According to Keen & Russell (1921), ground temperatures of 0° C. are the exception rather than the rule: during frost spells the ground may become frozen to a depth of several inches, but at other times, though the surface temperature frequently falls below zero, only the very superficial layer is frozen. Apart from periods of frost the temperature at a depth of 6 in. in the winter months showed an average daily range of about 2° C., around a mean value of about 4° C. Finally, Conway (1936) obtained ground-temperature data with a recording thermometer at Wicken Fen, Cambridgeshire, and found that the temperature on the soil surface frequently approached, but never reached, 0° C.

Discussion

The data quoted above suggest that soil temperatures above 30 or below about −1° C. do not regularly occur in England. If, therefore, the experimentally determined limits of tolerance be accepted as applicable to wireworms under natural conditions, it is seen that the limits of tolerance lie well within the normal range of environmental temperatures, and it must be concluded that wireworms in England are not normally liable to encounter lethal temperatures at any depth, and that extreme temperatures cannot be regarded as a factor of importance in limiting the vertical distribution of wireworms in the soil.

If, however, a more extended view is taken, and the extreme conditions which occur over a period of many years are considered, the picture is somewhat different. It seems from the records of soil temperatures that temperatures of 36° C. or more, which the experimental work has shown to be lethal, are likely to occur from time to time in the surface layers of the soil; while, during a sudden frost, a wireworm trapped near the surface of the soil might on rare occasions be subjected to a fall of temperature rapid enough to be fatal. The limits of tolerance of the wireworms seem, therefore, to correspond closely to the extremes of temperature to which the species is subject, though death from high or low temperatures must, in fact, be a rare event.

The environmental conditions found in other parts of the world inhabited by wireworms are, of course, very different, for the distribution of *A. lineatus* and *A. obscurus* extends from Siberia to the Mediterranean. In view of the close correspondence between the lethal temperatures of wireworms in England and the extremes of temperature which occur there, it must be expected that different physiological races having different degrees of resistance to extreme temperatures exist. That wireworms in central Germany and in Switzerland are considerably more resistant to low temperature than those used in the experiments described here is suggested by the work of Langenbuch (1932) and Guéniat (1937), to which reference has already been made. No information is available about the resistance of *Agriotes* from any part of the Continent to high temperatures, but experiments on the North American wireworm, *Melanothus communis*, showed its upper limit of tolerance to be about 46·5° C. (Fulton, 1928), which is 10° higher than that found for *Agriotes*. But *Melanothus* must probably be subjected to much higher temperatures than is *Agriotes* in England since, according to the same author, 'a temperature of 53° C. may be developed on the surface of partly dry sod ground when the air temperature in the shade is only 32° C.'

That the lethal temperatures of an animal are related to the environmental temperature has been shown by many investigations, but it is generally found that the lethal temperature lies well outside the normal range of environmental temperature and the animal appears to have a wide 'margin of safety'. The limits of tolerance of a species could not be expected to be extended much beyond the environmental extremes by means of selection, but if, as in the case of the wireworms, there is a close correspondence between the lethal temperature and the

extremes to which the species rather than the individual is subjected, then the adjustment of an animal's tolerance to the conditions of the environment by means of selection is more easily understandable.

II. ACTIVITY

The study of the effect of temperature on the activity of poikilotherms is complicated by the dependence of the metabolic rate on temperature. Since the metabolic rate is greater at higher temperatures, it is possible for the animal to perform its actions faster at higher than at lower temperatures. The amount of activity may, however, also be influenced by the animal's behaviour, since an animal does not necessarily spend all its time in performing any particular activity, and its behaviour will determine the proportion of time during which the animal is active. Though, from the practical point of view, it is probably the total amount of activity which is of importance, knowledge of how the animal's behaviour affects the continuity of its activity under different conditions is also important, particularly in connexion with feeding activity, for the following reason. If the amount eaten depends merely on the rate at which eating takes place, then temperature will affect the amount eaten in the same sort of way as it affects the rate of metabolism, and thus the ratio of food eaten to food used will, roughly speaking, be independent of temperature. If, on the other hand, temperature also influences the continuity of feeding activity, then the food eaten will be in excess of the food used when the feeding is most continuous. It seems probable, therefore, that the optimum conditions for feeding will best be indicated by measurement of the continuity of feeding.

The dual effect of temperature on activity raises another point of interest, in connexion with the avoidance of low temperatures. If the temperature falls to a low level, the speed of an animal's activity will fall correspondingly, till at some point cold stupor will set in and all movement cease. Unless the animal can escape from the region of low temperature before this occurs it will be trapped, and if the temperature continues to fall it may be killed. This can be prevented only if the behaviour of the animal in response to the fall of temperature maintains its activity at a high enough level to allow the normal reaction to operate, by which the animal keeps within the praeferendum. Such an effect has been recorded in blow-flies (Nicholson, 1934) and in *Ptinus tectus* (Gunn & Hopf, 1942). Since wireworms are probably liable occasionally to be subjected to lethal low temperatures if they remain near the surface of the soil (see Section I), it was of interest to discover whether an effect of this nature would be exhibited in their activity at low temperatures.

With some insects it is possible to assess the effect of behaviour on activity by observing in a sample of animals the number which are active at any particular moment (Nicholson, 1934; Gunn & Hopf, 1942). It was not possible, however, to make use of this method in the case of wireworms, since there was no clear-cut distinction between active and inactive individuals; when observed burrowing in sand between two glass plates, they showed all degrees of movement from almost imperceptible movements of the head up to vigorous burrowing. The plan adopted was therefore the following.

The effect of temperature on the speed of locomotion was determined under conditions which, by causing the wireworms to crawl continuously, ensured that behaviour did not influence the amount of activity. Then a form of activity, which was not necessarily continuous and which might therefore have been influenced by behaviour, was taken, and the amount of this activity was measured at different temperatures. By comparing the effect of temperature on this activity with the previously determined effect on the speed at which continuous activity took place, it was then possible to determine the extent to which the behaviour of the animals in this respect was influenced by temperature.

The forms of activity chosen for investigation in this way were burrowing and feeding, since these probably constitute the greater part of the wireworms' normal activity.

It was unfortunate that the work described here was carried out before the publication of the work of Evans & Gough (1942), which showed the feeding activity of wireworms to be periodic; large wireworms being found to feed for a few weeks and then to spend a rather longer period in fasting, toward the end of which they moulted. It is probable, therefore, that the animals used in the experiments described here included some in the feeding and some in the non-feeding states, and that the latter would show little activity in burrowing or feeding. Nevertheless, most of the wireworms used did in fact burrow and feed, though to a very variable extent, and the experiments provided the information that was sought.

Speed of locomotion

Technique

In order to make wireworms crawl continuously it is only necessary to place them on a surface which does not give them dorsal and lateral contact. For convenience in measuring their speed the animals must move along a definite track. This was made from a glass tube 40 cm. in length with an internal diameter of 0·8 cm. The 'floor' on which they crawled consisted of a layer of paraffin wax in which were imbedded small sand grains, the purpose of which was to give a rough surface on which the wireworms' legs could grip, so that all their locomotory activity was translated into motion. To ensure saturation of the air in the tube, a strip of moist filter paper was made to adhere to the roof, and the ends

were plugged with damp cotton-wool. The wax floor obviated the disturbing influence of free water which would have been present if the floor had been of damp filter paper.

The wireworms were introduced singly into one end of the tube, and their speed was measured over three consecutive distances of 10 cm., starting at a point 5 cm. from the point where crawling began. Observation was made by means of a dim red light kept vertically above the crawling animal.

Experiments were made on ten individual wireworms, which were kept between experiments in separate tubes of moist sand. Four experimental temperatures, 8, 15, 19 and 25°C., were obtained by working in three constant temperature rooms and a dark-room. Before each experiment the animals and apparatus were kept for at least 1 day at the experimental temperature, so that there could be no question of a reaction to a change of temperature. The experiments were done in May with freshly collected material. Only large larvae, measuring 19–23 mm. in length were used.

Results

The speeds of the individuals at each temperature are shown in Fig. 3, the points referring to each individual being connected by lines. The speed of each individual did not vary much over the course of 30 cm., and there was no consistent tendency for the

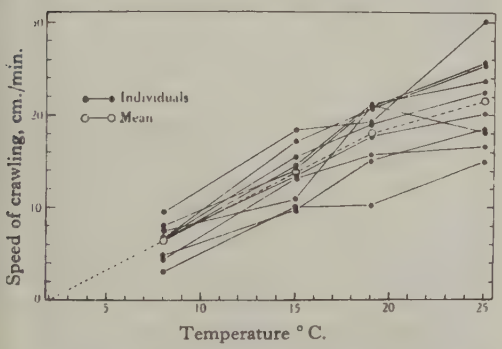

Fig. 3. Speed of crawling of ten wireworms, measured over a distance of 30 cm. at four temperatures. The points referring to each individual are connected by lines.

animals to speed up or slow down during the course: for this reason only the mean speed of each animal at each temperature is shown. Differences in speed between individuals were, however, considerable, but the animals maintained approximately the same order at each temperature (as can be seen from the tendency of the lines in Fig. 3 to run parallel). For this reason the line joining the mean values of all ten animals at each temperature represents the relationship between temperature and velocity of locomotion more accurately than would otherwise have been the case. The line is nearly straight within the limits of temperature covered by the experiment. A linear relationship of this sort is not usual, but it has been recorded by Herter (1928) for the leech *Hemiclepsis* and by Beauchamp (1935) for *Planaria alpina*. Assuming the relationship to be linear, the graph in Fig. 4 has been extrapolated to show the 'biological zero' or the temperature at which the velocity becomes nil, and this is seen to be about 2°C.

Burrowing activity

Technique

In order to measure their burrowing activity, the wireworms were placed in moist sand between two glass plates, and the length of burrow made by each in 1 hr. was measured. The burrows, which could be easily seen by transmitted light, were traced on to paper and measured with a map measurer. The sand in which the wireworms burrowed was sieved in order to obtain uniform particles between 0·5 and 1 mm. in diameter. The moisture content of the sand was adjusted to 20% of saturation, the amount of water required to saturate the sand having been previously determined by the addition of water to a known weight of sand in a tall cylinder till the surface was just covered. The glass plates measured 12 in. square and were separated by cardboard gaskets $\frac{1}{8}$ in. thick. Five pairs of plates with ten animals in each were used. Precautions were taken to ensure that the sand was evenly distributed throughout, so that the conditions should be as nearly as possible identical for each of the fifty animals in each experiment.

Experiments were made at temperatures of 6, 16, 24 and 33°C., of which the first three were obtained by working in three constant-temperature rooms and the fourth by the use of an incubator. The experiments at 33°C. were thus not strictly comparable with the others, since the plates could not be loaded and examined in the incubator.

In order that any effect of a change of temperature might be evident in the observations, the experiments were carried out in the following manner. Two successive experiments were first done at 24°C.; the animals had previously been kept for 2 weeks at this temperature, and thus no change of temperature was involved. The next experiment was done at 16°C., the animals being transferred directly from the 24°C. room to the apparatus at 16°. The first experiment at 16°C. thus recorded the activity of the wireworms after they had experienced a sudden fall of temperature from 24 to 16°C. A second experiment followed immediately which recorded the activity 1 hr. later. A third experiment at 16°C. was carried out on the following day, the wireworms having thus been previously kept for about 16 hr. at the experimental temperature. Three experiments were then

done in the same manner at 6°C., and after that the procedure was repeated at 16, 24 and 33°C., the temperatures being taken in ascending order.

Results

The burrowing activity of the wireworms in each experiment is shown in the form of a histogram in Fig. 4. In each histogram the lengths of the burrows are grouped into 2 cm. classes, and the number of burrows in each class is shown as a percentage of the total number of animals in the experiment. The

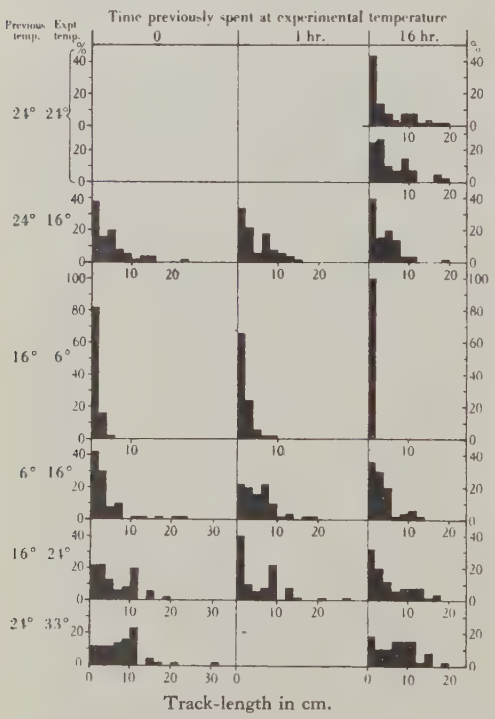

Fig. 4. Burrowing activity of fifty wireworms under various conditions of temperature. In each histogram the abscissa shows the length of burrow, grouped into 2 cm. classes, and the ordinate shows the percentage of burrows in each class. Further explanation in text.

effect of temperature on burrowing activity can best be seen from Fig. 5, in which the mean values of the activity shown by the animals in each experiment are given. The experiments are arranged in the order of performance, so that at each temperature (except the first and last of the series) there are three experiments differing in the period of previous exposure to the experimental temperature. Too much reliance should not be placed on the values shown in Fig. 5 on account of the great variation between individual wireworms. Moreover, the asymmetrical distributions of burrow lengths, shown in the histograms, renders an estimate of the accuracy of the mean values from their standard errors unreliable, and comparisons between the different experiments cannot be made with any confidence. Nevertheless, the following conclusions seem to be justified: burrowing activity increased with rise of temperature up to the highest temperature used, and there was no indication of any intermediate 'optimum' temperature; the experiment did not suggest that the effect of temperature on burrowing activity differed from that on the speed of continuous crawling.

The point of greatest interest brought out by this experiment concerns the activity at low temperatures. It can be seen, particularly from Fig. 4, that the activity at 6°C. appears to be much less after 16 hr. at that temperature than immediately after the temperature had been lowered from 16°C. If this differ-

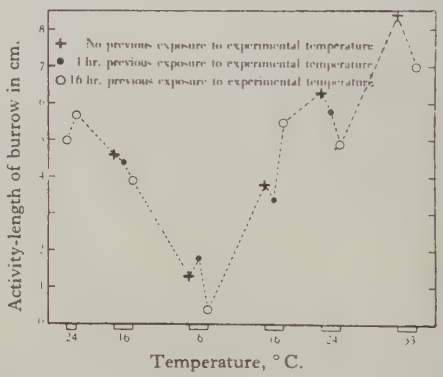

Fig. 5. Mean length of burrow made by fifty wireworms under various conditions of temperature. Further explanation in text.

ence were real, it would indicate a behaviour effect of interest in connexion with the animals' mechanism of avoiding low temperatures. It is therefore important to know whether the differences between the results of the experiments at 6°C. are significant. Since the usual statistical procedure would not be reliable if applied to asymmetrical distributions of this nature, a means of expressing the activity of the animals in such a way as to give a distribution approximating more closely to a normal curve was sought. It was found that this could be effected by expressing the burrowing activity of each animal in terms of the square-root of the length of burrow made by it. When this was done the following mean values (with their standard errors) were obtained:

(1) No previous exposure to 6°C.: $M = 0{\cdot}94 \pm 0{\cdot}075$.
(2) 1 hr. previous exposure to 6°C.: $M = 1{\cdot}13 \pm 0{\cdot}100$.
(3) 16 hr. previous exposure to 6°C.: $M = 0{\cdot}38 \pm 0{\cdot}063$.

Statistical comparison of the first and last experiments shows the difference in activity to be significant. (The difference between the two means is 5·6 times the standard error of the difference.) This means that after the temperature had been reduced from 16 to 6°C. the wireworms' activity was significantly greater during the first 2 hr. at 6°C. than it was 16 hr. later. Moreover, the activity after 16 hr. at 6°C. was almost nil, and it may be supposed that if the activity were immediately reduced to this level and a lethal temperature were subsequently reached, there would be no means of escape. A delay in the reduction of activity following a fall of temperature, such as was evident in the experiments, would, however, enable the wireworms to move into a more favourable region.

Feeding activity

Technique

The feeding activity of wireworms was measured by the use of wheat grains as food, the amount eaten in a given time being determined by weighing the grains before and after the experiment. Two series of experiments were performed and the technique differed in detail according to whether the wireworms were treated individually or in batches. The individual treatment made it possible to assess the feeding activity only from those individuals which did feed, whereas when larger numbers of animals in batches were used, the number of animals which actually fed was not known.

In the *first series* the feeding activity of ten wireworms was measured individually at eight experimental temperatures. Each animal was put, together with one wheat grain, in a 2 in. × ½ in. glass tube containing moist sand (moisture content, 20% of saturation). Small tubes were used in order to reduce as far as possible the time which a wireworm might spend in locating the grain before it commenced feeding. The wheat grains, which were dead and did not germinate, were soaked in water for 5 days before each experiment. They were then superficially dried and weighed individually, and at the end of the experiment, which lasted in every case for 3 days, they were reweighed. When a wireworm attacked a wheat grain it bored into it, sometimes eating out the entire contents of the grain, sometimes emerging at the other side leaving a tunnel through the grain. In any case a certain amount of the soft endosperm material oozed from the tunnel and embedded the surrounding sand grains. Before the grains were weighed, all the adhering sand was brushed off, and this included, especially with the more heavily attacked grains, a certain amount of endosperm material which was not in fact eaten. The measurements thus tended to exaggerate the feeding activity roughly in proportion to the amount actually eaten.

Control experiments, without wireworms, were carried out at each experimental temperature, and in calculating the weight eaten by the wireworms in the experimental series, appropriate allowance was made for the changes of weight undergone by the wheat in the controls (never more than 2% of the original weight). In order to standardize the state of hunger as far as possible, and to eliminate possible effects of changes of temperature, the animals were kept at the experimental temperature for a week before the experiment and were provided with wheat grains for food. No animal was used in more than one experiment.

In the *second series* of experiments larger numbers were employed and observations were made at each of five different temperatures on fifty animals divided into five batches of ten. Each batch was put into a small glass jar measuring 2 in. × 2 in., and was given fifty wheat grains. There were in this case about three times as many wheat grains per unit volume of sand as in the first series of experiments, and the possibility of a delay in the wireworms finding the food was consequently even less than before. The wheat used in these experiments was soaked in water for 2 days before use: it was living and started to germinate during the experiments at intermediate temperatures. For this reason the wheat in the control experiments gained in weight to a greater extent than before (from 6 to 10% of the original weight according to the temperature); but since the grains were weighed in batches of fifty, any variation which there may have been probably did not impair the reliability of the experimental results.

The technique employed in the second series of experiments gave the animals more freedom of movement and also enabled them to feed from more than one wheat grain. Thus, by counting the number of grains which had been attacked, as well as measuring the weight eaten, it was possible to estimate the continuity of the feeding activity. For, if the animals fed continuously, without moving about, much would be eaten from a few grains and the ratio of amount eaten to number of grains attacked would be high; whereas a low ratio would indicate that feeding had been interrupted, the animals having spent more time in moving about.

The experiments were designed to show also whether feeding activity was influenced by a change of temperature. Each batch of animals was accordingly kept at a constant temperature of 12°C. for a week before the experiment (wheat being provided as food), and then transferred directly to the experimental temperature. At each temperature three consecutive experiments, each lasting 2 days, were performed. Thus a comparison could be made between the feeding activity immediately after a sudden rise of temperature from 12°C. to the experimental temperature, with that after 2 and 4 days spent at the temperature of the experiment.

In all other points the technique was the same as that of the first series of experiments.

Results

The results obtained from the first series of experiments are given in Fig. 6. The feeding activity is shown as the mean of the weights eaten by the animals which fed (the number is shown beside each point), those which did not attack the wheat being excluded from the evaluation. The feeding activity shows a fairly regular increase from 11 to 32° C. and there is no clear indication of an intermediate optimum temperature. At the two extreme temperatures (7·5 and 34° C.) few of the animals fed and the amount which they ate was small. This was to be expected in view of the fact that burrowing activity was shown by the experiments described in the previous part of this paper to be almost nil at 6° C., while 34° C. is only about 2° below the lethal temperature of wireworms (see Section I).

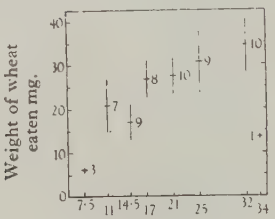

Fig. 6.

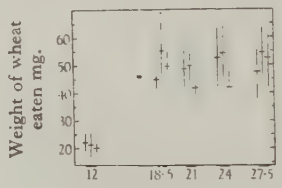

Fig. 7.

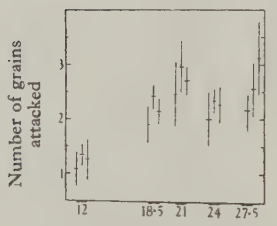

Fig. 8.

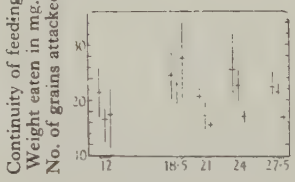

Fig. 9.

Figs. 6, 7, 8, 9. Feeding activity of wireworms at different temperatures. Each point indicates the activity per wireworm per day, and the vertical lines extend to ± twice the standard error. Further explanation in text.

The results of the second series of experiments, which refer to a rather narrower range of temperatures, are set out in Figs. 7–9. In these experiments it was impossible to distinguish the individuals which fed from those which did not, and the mean values of the feeding activity are each based on five observations of the combined feeding of ten animals. The chief interest in these results is contained in the records of the continuity of feeding activity expressed in Fig. 9 as the ratio of amount eaten to the number of grains attacked. If the third experiments at each temperature (i.e. after 4 days at the experimental temperature) are compared, it is seen that the ratio is significantly higher at 18·5° C. than at all higher temperatures, and is probably higher than at 12° C. This means that feeding was most continuous at 18·5° C. Thus, if continuity of feeding indicates optimum conditions, the results indicate an optimum temperature for feeding at about 18° C.

Comparison of the three experiments at each temperature shows that there are significant differences in the continuity of feeding between the first and last experiment at the three highest temperatures, feeding being less continuous after the fourth day at the experimental temperature. This suggests that the effect of a rise of temperature on the continuity of feeding is not immediate, but is manifest only after about 4 days.

III. TEMPERATURE PREFERENCE

In their natural habitat in the soil, wireworms are subject to considerable fluctuations of temperature both seasonal and daily. There is, moreover, generally a vertical gradient of temperature in the soil which varies in a rather complicated manner throughout the day; but the soil tends to be warmer nearer the surface in summer and colder nearer the surface in winter (Tutin, 1928). It is therefore possible that wireworms exhibit a 'preference' for a particular

range of temperature, and that their distribution in the soil might be controlled thereby, as indeed several authors have supposed to be the case (see review by Thomas, 1940). The following analysis of the reactions of wireworms to differences of temperature in their medium was therefore undertaken with the object of showing whether the wireworms exhibited such a preference or not. As was pointed out at the beginning of this paper, however, it is not possible on the basis of this work alone to predict the movements of wireworms under natural conditions.

The only previous work of this nature dealing with *Agriotes* (Deal, 1941) is inconclusive. The animals were placed in a trough of soil along which there was a gradient of temperature, and they were found to aggregate near the cold end of the apparatus at a temperature of 5–8°C. It is probable, however, that an aggregation of this sort does not necessarily indicate a preference for low temperatures, but is simply the result of the reduced activity at low temperatures.

Technique

The method of the 'alternative chamber' was adopted, since the use of a gradient apparatus has the disadvantage, for work on wireworms, that it is not possible to exclude from the observations those individuals which are totally inactive. The chamber consisted of a glass tube 1 in. in diameter and 10 in. in length. One half was kept at air temperature in one or other of three constant-temperature rooms and the other half was heated by immersion in a water-bath controlled thermostatically and stirred by compressed air. The arrangement is shown in Fig. 10. Four similar tubes were used concurrently, two as controls and two as shown in the diagram. It will be seen that the water heated the lower half of one of these tubes (*A*) and the upper half of the other (*B*). The use of four separate tubes set up vertically made possible an analysis of any gravitational response to temperature conditions that might exist.

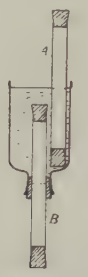

Fig. 10. Diagram of alternative-chamber apparatus.

The tubes were filled with loose peaty 'fen' soil, which was found to be better for the purpose than sand, as it allowed freer movement of the wireworms. Before use it was passed through a sieve of $\frac{1}{10}$ in. mesh. The water content was determined by drying samples at intervals throughout the course of the experiments. At the beginning it was 80% and at the end 66%. At the boundary in the middle of each tube a cross-wire was fixed which allowed the two halves to be emptied separately, but did not impede the movements of the wireworms. At the end of each trial the soil in each half of the four tubes was tipped out separately, and the number of animals in each half was counted.

Thirty wireworms were used in each tube and were placed all together about 1 in. from one or other end of the tube. In one experimental tube the animals were placed in the upper half, while in the other, in which the temperature conditions were reversed, they were placed in the lower half. In the control tubes the animals were placed in the upper end of one and the lower end of the other. Both were at a uniform temperature equal to that into which the animals in the experimental tubes were placed.

The temperature of the soil in the tubes, and of the room in which the animals were handled, was always the same as the temperature into which the animals were placed, so that they were not subjected to a sudden change of temperature at the beginning of the experiment. In most cases the animals were left for 2 days in the apparatus before examination, but a few trials were made which lasted only 1 day.

The following temperatures were used in various combinations as alternatives: 6, 11·5, 17, 21, 24, 30, 34°C. It was found, however, that at 6°C. the animals were too inactive to give clear responses, and no results are quoted for this temperature.

The temperature conditions in the two experimental tubes were examined by means of a thermocouple, when the air and water temperatures differed by 10°C. It was found that the gradient in the middle of the tubes was steep, a change of 6°C. being registered over the middle 2 in.; but the gradient continued, less steeply, almost up to the ends of the tubes.

Since there were always a number of animals which were inactive, it was not possible to interpret the results on the basis of the deviation from a random distribution. (In fact the control tubes seldom showed a random distribution.) The results were accordingly treated in the manner adopted for similar work on soil moisture by Lees (1943*b*). Comparisons were made of the number of animals migrating in the experimental tubes from the original temperature into the alternative, with the number migrating in the control tubes from the same original temperature into the other end of the tube at the same temperature. If the number migrating in the experimental tube was less than that in the control, it indicated an avoidance of the alternative temperature. The inactive animals were thus not involved in the observations, and their numbers did not affect the results, provided the effects of possible differences

between the batches were eliminated. In order to obtain a test of significance, four or eight replicate tests were made with each pair of alternative temperatures.

It is important to notice that the method of analysis tested the avoidance only of the alternative temperature and not of the original one, for if the original temperature was the one which was avoided, the response was generally obscured by those inactive animals which did not move from their original position. (See, for example, the first row of Table 3.) In such a case preference for the alternative temperature would be revealed only if the number of animals found in the original half of the experimental tube were significantly less than 50%: this occurred only in one set of tests (see last row of Table 3).

Throughout the whole series of experiments four separate batches of thirty wireworms were used. In the replication of each experiment the batches were alternated between the experimental and control tubes, in order to eliminate from the comparisons the bias due to any differences that there might have been in the number of inactive animals contained in each batch. Moreover, all the replicates of any experiment were made at about the same date, so that the validity of the comparisons should not be affected by replacing from stock the animals which died from time to time throughout the experiment. The material was obtained at the end of October, and the experiments were done during the winter and early spring, starting in November and finishing in March.

Results

1. *Gravitational responses.* Two possible gravitational responses must be considered. The animals might react to a uniform 'unfavourable' temperature by upward, or, more probably, downward movement. Alternatively, they might react in the same way only when subjected to a change in temperature, as when crossing the boundary. Analysis of the first of these possibilities involves comparison of the two control tubes at each temperature, while the second requires comparison of the two experimental tubes for each pair of alternative temperatures. These two sets of comparisons are set out in Table 2, from which it can be seen that under no set of temperature conditions was there any evidence of a gravitational response. This result is in agreement with the conclusion drawn by Lees (1943*b*) that burrowing wireworms do not respond to gravity.

Table 2. *Analysis of gravitational response. Number of wireworms migrating (out of a total of 30) upwards and downwards under various conditions of uniform temperature and of temperature change at the boundary*

Temp. of start °C.	Change at boundary °C.	Direction of movement	No. of exps.	No. migrating		*t*	*P*
				Mean	Standard error		
11	0	Down	4	8·25	2·87	0·3	> 0·1
11	0	Up	4	9·0	3·05		
17	0	Down	4	14·75	4·80	0·6	> 0·1
17	0	Up	4	12·5	5·00		
17	0	Down	8	10·5	2·76	1·1	> 0·1
17	0	Up	8	7·25	2·03		
24	0	Down	4	11·0	3·24	0·4	> 0·1
24	0	Up	4	10·75	3·64		
11	+ 6	Down	4	7·0	2·70	0·3	> 0·1
11	+ 6	Up	4	6·5	2·29		
17	− 6	Down	4	4·75	1·58	0·6	> 0·1
17	− 6	Up	4	6·25	2·12		
17	+ 4	Down	4	6·5	2·18	0·1	> 0·1
17	+ 4	Up	4	6·25	2·18		
24	−13	Down	4	8·75	2·70	0·5	> 0·1
24	−13	Up	4	10·5	3·46		

2. *Temperature responses.* The absence of a gravitational response allows the orientation of the tubes to be ignored in analysing the temperature responses. The results are set out in Table 3. In the first two columns the various combinations of temperatures used as alternatives are given, that on the left being the temperature in which the animals were initially placed. All the differences are clearly significant, and the results shown in the last two columns indicate that a temperature of 17°C. is preferred to 11·5°C. and to 21°C. and all higher temperatures.

This does not, of course, define the temperature praeferendum within at all narrow limits. It does,

however, show clearly that the wireworms have a definite reaction to temperatures both above and below their praeferendum.

3. *Effect of previous temperature.* Since the method used in the above experiments demonstrated only the avoidance by the animals of the alternative temperature, it might be argued that their preference was entirely dependent on the previous temperature, their reaction being to avoid any change of temperature.

The results shown in the first and last rows of Table 3, however, make it clear that this was not the case when the previous temperature was 11·5 or 34°C., but the possibility is not disproved for intermediate temperatures. A small experiment was accordingly carried out in order to test this point.

Temperatures of 11·5 and 24°C. were chosen as alternatives because these were respectively below and above the praeferendum indicated by the experiments and they therefore offered a sharp contrast without involving any clear preference. The animals were exposed for 4 days to a temperature of 24°C., and then the trials were made by starting the animals at the 24°C. ends of the tubes, thus testing for avoidance of the lower temperature. The results were as follows, means being based on eight trials each.

Mean no. migrating from 24°C. to 11·5°C. = 9·6 ± 1·55
Mean no. migrating in control at 24°C. = 10·9 ± 1·70

Statistical comparison of the means gives $t = 1·1$, for which P is $>0·1$. The difference is very small, and the conclusion is that 4 days' exposure to 24°C. did not cause the animals to avoid 11·5°C. It would seem, therefore, that the temperature preference revealed by the experiments was not in fact dependent on the previous temperature.

Table 3. *Temperature preference of wireworms by alternative-chamber method. Number migrating (out of total of thirty) from the temperature shown on the left into that shown on the right of the first column*

Alternative temperatures °C.		No. of exps.	No. migrating		t	P	Temperature preference	
			Mean	Standard error			Selected	Avoided
11·5	17	8	6·8	3·01	—	—	—	—
11·5	11·5	8	8·6	1·60				
17	11·5	8	5·5	1·00	3·9	<0·01	17	11·5
17	17	8	13·6	2·57				
17	21	8	6·4	1·14	3·3	<0·01	17	21
17	17	8	11·3	1·96				
24	30	7	6·7	1·60	2·6	0·02	24	30
24	24	8	12·4	2·39				
					χ^2			
34	24	7	19·7	3·26	9·2	<0·01	24	34

Mechanism of response

The experiments described above showed that burrowing wireworms avoided temperatures above and below certain limits. The mechanism of orientation whereby this response is brought about may now be considered.

It is clearly possible that the avoidance of high temperatures may have been due simply to the increased rate of movement at high temperatures, which was demonstrated in Section II. That this mechanism—orthokinesis, in the terminology of Fraenkel & Gunn (1940)—was not solely responsible for the reaction will be shown by the experimental results presented below. The avoidance of low temperatures, however, could not have been due to orthokinesis, since the rate of movement was never found to be increased by a fall of temperature.

Moreover, it is generally agreed that under natural conditions wireworms live in systems of semi-permanent burrows in which they move about through the soil (Roberts, 1919; Rothamsted *Report*, 1938–9; Lees, 1943*b*). Being thus restricted in their movements, the only mechanism of orientation, other than orthokinesis, by which the wireworms could react to differences of temperature, is by stopping in their tracks when they encounter unfavourable conditions and backing down the burrow. That wireworms respond to differences in humidity by means of a 'shock reaction' of this sort has been demonstrated by Lees (1943*a*). Direct evidence of this mode of response was, however, not sought in the present work owing to the difficulty of constructing an artificial burrow with a temperature boundary, and excluding the possibility of there being small differences in humidity, to which wireworms are highly reactive (Lees, 1943*a*).

New burrows must, however, sometimes be made, and the wireworms, while moving freely through the soil, might react to unfavourable conditions of temperature by changing their direction of movement. Only a mechanism dependent on the rate of change of direction (i.e. klinokinesis) need be considered, since any mechanism which depends on the differential stimulation of the two sides of the body (i.e. a taxis) could hardly be brought about by gradients of temperature such as exist in the soil.

There were thus two questions to be answered experimentally: first, whether the avoidance of high temperatures was due solely to orthokinesis, and secondly, whether wireworms engaged in making new burrows responded to differences in temperature by klinokinesis.

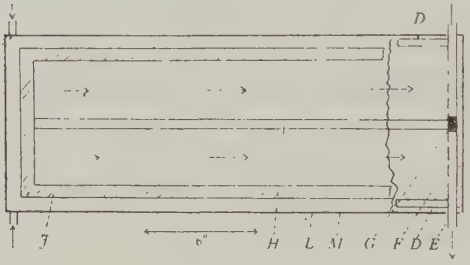

Fig. 11*a*.

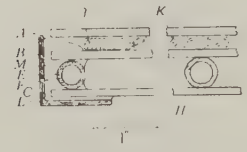

Fig. 11*b*.

Fig. 11. Diagram of alternative-chamber apparatus for observing movement of wireworms in sand. (*a*) Plan: on the right the upper plates have been removed. The arrows indicate the flow of water. (*b*) Sections through end, and across centre of apparatus. *A*, *B*, *C*, glass plates. *D*, glass tubes, separating *B* and *C* along sides. *E*, brass tubes, separating *B* and *C* along ends. *F*, holes in tubes (*E*) for entry and exit of water. *G*, cork, plugging middle of tubes (*E*). *H*, rubber tube between *B* and *C*, separating the two streams of water. *J*, rubber gasket, separating *A* and *B*. *K*, sand. *L*, angle-iron frame. *M*, cement, made of red-lead and glycerine.

Technique

An alternative-chamber apparatus was constructed in which the tracks of wireworms burrowing in sand could be recorded in the manner described in Section II. In this case the glass plates were larger (24 in. × 9 in.), and the lower one formed the upper wall of a chamber through which circulated two streams of water, separated by a partition down the middle of the chamber. The construction of the apparatus is shown in Fig. 11 (*a*), (*b*). One stream of water was heated before entering the chamber, and thus a constant temperature difference was maintained between the two halves of the chamber in which the wireworms burrowed. The temperature of the two parts was recorded by thermometers in the two entering streams of water. The temperature conditions in the sand were investigated by means of a thermocouple, and are shown in the form of isotherms in Fig. 12. The gradient of temperature across the boundary was steep—2·8° C. per in. when the difference between the two streams of water was 5° C.; and 6° C. per in. when the difference was 10° C. Moreover, the temperature was very uniform at corresponding points along the length of the chamber, and the apparatus is considered to have been a satisfactory form of alternative chamber for observing the movement of animals in sand.

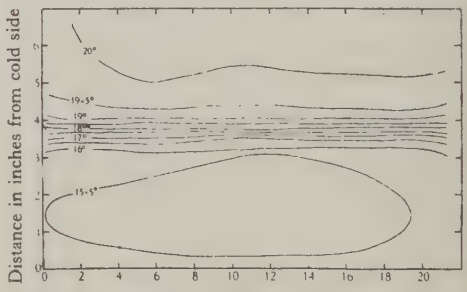

Fig. 12. Temperature of sand in alternative-chamber apparatus. Isotherms drawn at 0·5° C. intervals.

Wireworms were placed singly in the sand along the centre of the apparatus, and each animal was isolated from its neighbours by a glass rod running across the chamber. The sand was sieved, as before, and its moisture content adjusted to 20% of saturation.

Results

A number of observations were made with various temperature differences, and representative examples of tracks are shown in Fig. 13. The nine tracks on the left of the figure were made when the temperatures in the two halves of the chamber were 14 and 24° C., and the eight tracks on the right when the temperatures were 16·5 and 26·5° C. In each case the animals were left in the apparatus for 9 hr. before the tracks were recorded. Temperatures below about 14° C. (that of the tap water) were not employed, and therefore in all the tests it was the higher temperature which the wireworms were expected to avoid.

If the avoidance of high temperatures were due solely to orthokinesis, it would be expected that the tracks would be equally divided between the two halves of the chamber, and the animals would aggregate in the cooler side simply on account of their slower movement there. It was found throughout the tests, however, that most of the tracks were made on the cool side only, and so the avoidance of high temperatures cannot have been due solely to orthokinesis.

Again, if klinokinesis were the mechanism of the wireworms' reaction, the tracks on the two sides of the chamber would be dissimilar, and those on the warm side would be more convoluted owing to the greater rate of change of direction. No indication of such a difference was found in any of the tests, and the tracks appeared entirely similar at all temperatures. It must therefore be concluded that burrowing wireworms do not react to differences of temperature by klinokinesis.

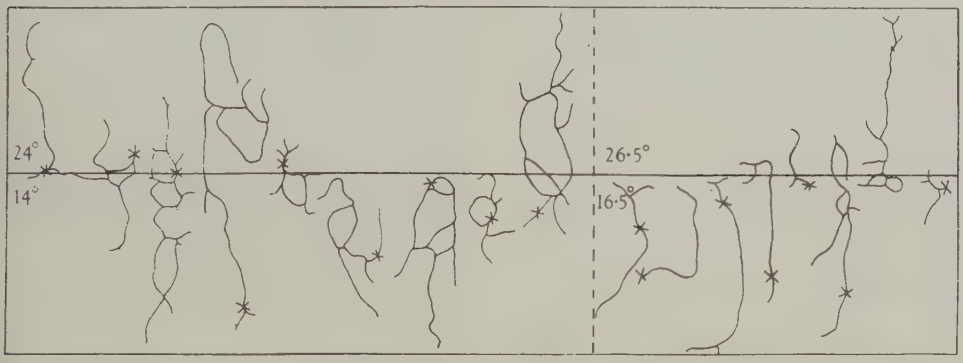

Fig. 13. Tracks made by seventeen wireworms in alternative-chamber apparatus. The final position of each animal is marked by a cross. Further explanation in text.

Since neither orthokinesis nor klinokinesis was the mode of response, it appears that the avoidance of unfavourable temperatures must be brought about by a 'shock reaction'; that is to say, on encountering unfavourable conditions, the animal completely reverses the direction of its movement, and crawls backward down the burrow. Direct evidence of this reaction was not obtained (for the reason stated above), but it was frequently noticed that burrows stopped short at, or just beyond, the boundary, and thus it seems probable that the shock reaction operates both when the animals are moving in existing burrows and when they are engaged in making new burrows.

SUMMARY

I

1. The highest temperature which wireworms could withstand for an indefinite period without becoming paralysed was found to be between 35 and 36° C.

The resistance to high temperature was not influenced by the previous temperature.

2. The resistance of wireworms to low temperature was found to be greatly influenced by the rate at which the temperature was reduced.

When the temperature fell rapidly, all were killed by 6 days' exposure to −3° C., and by 4 hr. exposure to −7° C.

The resistance to low temperature was not increased by a previous exposure of 24 hr. at 0° C., when the subsequent fall of temperature was rapid.

When the temperature was lowered in small stages, however, the resistance was considerably increased, and a few wireworms survived for at least a day at −10° C.

3. The published data of soil temperatures suggest that temperatures above 30° C. or below about −1° C. are not of regular occurrence in England, and it is concluded that wireworms in England are not normally liable to encounter lethal temperatures.

II

4. The relationship between the wireworms' speed of crawling and the temperature was found to be nearly linear between 8 and 25° C.

5. Burrowing activity was uniformly greater at higher than at lower temperatures between 6 and 33° C. Temperature influenced the speed but not the continuity of the activity.

After adaptation had taken place the burrowing activity was almost nil at 6° C.

When subjected to a sudden fall of temperature from 16 to 6° C., the wireworms' burrowing activity in the first 2 hr. was significantly greater than it was at the same temperature 16 hr. later.

6. The weight of wheat eaten by wireworms was found to be greatest at 32° C., but feeding activity

was most continuous at about 18°C. At 7°C. and at 34°C. little feeding took place.

III

7. No vertical movements in response to gravity were exhibited by the wireworms under any conditions of temperature.

8. When given a choice of temperatures in an alternative-chamber apparatus, the wireworms showed a clear preference for 17°C. as against 11·5 and 21°C. or higher temperatures.

The temperature preference was not found to be influenced to any great extent by the previous temperature.

9. The mechanism by which the wireworms reacted at a temperature boundary was of the nature of a 'shock reaction', their direction of movement being reversed.

No evidence of a klinokinetic response to change of temperature was obtained from wireworms burrowing in sand.

I should like to express my thanks to Prof. J. Gray, F.R.S., for accommodating me in his laboratory, and to all those who have given me advice throughout the course of the work.

REFERENCES

BEAUCHAMP, R. S. A. (1935). *J. Exp. Biol.* **12**, 271.
BLISS, C. I. (1935). *Ann. Appl. Biol.* **22**, 134.
BLISS, C. I. (1937). *Ann. Appl. Biol.* **24**, 815.
CONWAY, V. M. (1936). *New Phytol.* **35**, 359
DEAL, J. M. (1941). *J. Anim. Ecol.* **10**, 323.
EVANS, A. C. & GOUGH, H. C. (1942). *Ann. Appl. Biol.* **29**, 168.
FRAENKEL, G. S. & GUNN, D. L. (1940). *The Orientation of Animals.* Oxford.
FULTON, B. B. (1928). *J. Econ. Ent.* **21**, 889.
GUÉNIAT, E. (1937). *Mitt. schweiz. ent. Ges.* **16**, 167.
GUNN, D. L. & HOPF, H. S. (1942). *J. Exp. Biol.* **18**, 278.
HERTER, K. (1928). *Z. vergl. Physiol.* **7**, 571.
KEEN, B. A. & RUSSELL, E. J. (1921). *J. Agric. Sci.* **11**, 211.
LANGENBUCH, R. (1932). *Z. angew. Ent.* **19**, 278.
LEES, A. D. (1943*a*). *J. Exp. Biol.* **20**, 43.
LEES, A. D. (1943*b*). *J. Exp. Biol.* **20**, 54.
MELLANBY, K. (1939). *Proc. Roy. Soc.* B. **127**, 473.
MELLISH, H. (1899). *Quart. J. R. Met. Soc.* **25**, 238.
NICHOLSON, A. J. (1934). *Bull. Ent. Res.* **25**, 85.
Radcliffe Observations. *Results met. Obsns. Radcliffe*, **55**, 102, 104.
ROBERTS, A. W. R. (1919). *Ann. Appl. Biol.* **6**, 116.
Rothamsted (1938–9). *Soil insecticide investigations report.*
RUSSELL, E. J. (1937). *Soil Conditions and Plant Growth.* London.
THOMAS, C. A. (1940). *The Biology and Control of Wireworms.* State Coll. Pennsylvania.
TUTIN F. (1928). *Report on Agricultural Meteorological Conference.* Min. of Agric. and Fisheries.

ON THE MOVEMENT OF WIREWORMS OF THE GENUS *AGRIOTES* ESCH. (COLEOPTERA, ELATERIDAE) ON THE SURFACE OF THE SOIL AND THEIR SENSITIVITY TO LIGHT*

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(*Received* 5 *August* 1944)

(With Four Text-figures)

During the course of experimental work on the behaviour of wireworms in relation to temperature (Falconer, 1945), stocks of wireworms were kept in sand in glass jars, and it was often noticed after a jar had been left undisturbed for some time that the surface of the sand was pierced by many small holes of the size of a wireworm burrow. This fact suggested that, contrary to general belief, wireworms might sometimes come out on to the surface of the soil and wander about before re-entering, perhaps at a different point. If this were the case, the fact might have considerable importance in connexion with migration and possibly with food finding. Reference to the matter is scanty in the literature. Subklew (1934) stated that though the larvae are well adapted to life in the soil, they are also able to crawl about extensively on the surface: in a later paper, however (Subklew, 1935), he concluded that a wireworm which had come out on to the surface would at once be led below ground again by its thigmotactic response. Guéniat (1937) speaks of collecting abundant wireworm material in early spring without digging, the larvae being found under stones and lumps of earth: these larvae, he says, eat young grass shoots and dandelion leaves. Though it is not explicitly stated, his remarks strongly suggest that wireworms in Switzerland habitually move about on the surface of the soil in early spring. There does not, however, appear to be any evidence of similar behaviour on the part of wireworms in England.

The problem appeared to be an interesting one, and experiments were accordingly carried out with the object of finding out whether the wireworms in the laboratory did in fact crawl about on the surface, and what factors influenced their emergence and the point of their re-entry.

The wireworms used were obtained from fields in Cambridgeshire. Only medium- to large-sized larvae were used, and the majority were probably *A. lineatus* or *A. obscurus*, though a few large individuals of *A. sputator* may sometimes have been included.

To obtain records of surface movement a rectangular glass dish, measuring 10½ in. × 8½ in. × 2½ in. deep, was divided into six compartments by vertical glass partitions reaching to a level of ½ in. below the top of the dish. The dish was filled with moist sand† to the level of the top of the partitions and the whole covered with a glass plate made airtight by plasticene. Fig. 1 shows this apparatus without lid or sand. The numbers in the figure refer to the compartments. Except where otherwise stated, the dish was kept in the dark.

A number of wireworms were put in one or more of the compartments (approximately in the centre of the compartment) and after one or more days the numbers in all the compartments were counted. Since a wireworm could change compartments only by crawling over the surface, the method gave a means of measuring the numbers exhibiting surface movements under different conditions. If a wireworm moved only a short distance over the surface before re-entering, it would be more likely to enter the compartment from which it came than a different one. In fact it was observed that the animals moved about quite extensively on the surface, and since the surface area of each compartment was relatively small the likelihood of a wireworm re-entering the same

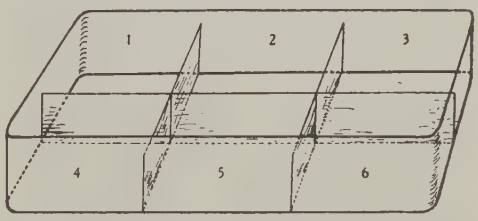

Fig. 1. Sketch of apparatus used for recording surface migration. Glass lid and sand not shown.

* The work was performed during the tenure of a studentship from the Carnegie Trust for the Universities of Scotland.

† The moisture content of the sand was 20% of saturation, the amount of water required to saturate the sand having been determined by adding water to a known weight of sand in a tall cylinder, till the surface was just covered. The sand was sieved so as to obtain uniform particles 0·5–1·0 mm. in diameter.

compartment in which it came to the surface was scarcely greater than the likelihood of it crossing a partition into a neighbouring compartment.

FACTORS INFLUENCING EMERGENCE

Twenty wireworms were put in each of three compartments (nos. 1, 3 and 5) and at the end of each experiment the total number found in the other compartments (nos. 2, 4 and 6) gave a measure of the number which came to the surface. The actual number which emerged must have been approximately twice the number recorded, for an equal number would have re-entered the original three compartments. Six experiments, designed to show whether the wireworms did migrate over the surface or not, were first performed. Each experiment lasted 1 or 2 days. The total numbers of animals found in the new compartments (nos. 2+4+6) after the experiments were 13, 13, 12, 17, 8 and 14. The results left no doubt that surface migrations did take place; in fact an average of about 43% of the larvae must have come to the surface in each period of 24 hr.

Effect of light. Four experiments were done with a light, consisting of a 60-watt lamp, placed 12 in. above the surface of the sand. A water screen was placed above the dish to prevent the sand from being heated. The total numbers of wireworms found in the new compartments after these experiments were 16, 18, 16 and 9. It is clear, therefore, that the illumination made no significant difference to the number of animals which emerged. This fact is interesting on account of the known reactions of wireworms to light, and the matter will be taken up in greater detail below.

Effect of humidity. Wireworms were known to react to very small differences in the humidity of the air, and to avoid dry conditions (Lees, 1943*a*). One experiment was accordingly carried out with the lid of the dish removed, so that the surface of the sand was exposed to air, which, with a relative humidity of 85%, was comparatively dry. No wireworm was found in the new compartments, and the experiment showed, therefore, that relatively dry air prevented the wireworms' emergence.

Effect of food in the sand. In none of the above experiments were the larvae provided with food. Three experiments were carried out in the same manner, but abundant food, in the form of soaked, dead wheat grains, was provided. It was mixed with the sand in all compartments, and care was taken that no grains should be actually on the surface. The total numbers found in the new compartments were 1, 3 and 4. The surface migration was thus shown to be greatly reduced by the presence of food in the sand.

In order to confirm this result an experiment was set up in which the effect of the presence of food would be cumulative. Food was mixed with the sand in compartments 2, 4 and 6, and the wireworms were put without food in the other three compartments (fifteen in each). At various intervals up to 13 days after the start, the numbers in the different compartments were counted and the wireworms returned to the same compartment in which they were found. Table 1 shows the results. The first five observations (up to the 8th day) were made by counting only the no-food compartments, the numbers which had migrated being obtained by subtraction from the total. The number of wireworms in the food compartments increased steadily during this period. On the 8th and subsequent days, in order to make sure

Table 1. *Surface migration of wireworms leading to an aggregation in compartments with food. The fourth column shows the total number of animals, excluding those which died in the no-food compartments without having migrated*

Duration: days from start	Nos. counted		Total	% of total in compartments with food
	Without food (living)	With food (living + dead)		
0	45	0	45	0
1	24	—	37	35
2	22	—	36	39
5	12	—	36	67
6	9	—	35	74
8	7	24	31	77½
9	—	22	31	71
10	—	24	31	77½
13	—	24	31	77½

that the steady migration was not caused by the periodic disturbance of the wireworms in the no-food compartments, the observations were made by counting the numbers found in the food compartments, the others being left undisturbed. The numbers, however, were found to remain substantially constant, and the disturbance cannot, therefore, have been an important factor in causing the migrations. Throughout the experiment there was rather a high mortality. When this occurred in the original, no-food, compartments the dead animals were subtracted from the total: these amounted to fourteen in the first 8 days. But those found dead in the compartments with food were not deducted from the total since they had already exhibited surface movement. These amounted to five in the first 8 days and one between the 10th and 13th day. None died in the no-food compartments after the 8th day. Throughout the course of the experiments there was a marked aggregation of wireworms in the compartments with food. Since the food was entirely below the surface, it is improbable that its presence in any compartment favoured the re-entry of wireworms in that compartment, and the conclusion that the presence of food reduced the number of wireworms which emerged was, therefore, confirmed.

Is coming to the surface a directed movement? The comparatively high proportion of animals which were recorded in the above experiments as having come to the surface raised the question of whether the movement was directed or merely random. Though it did not seem likely that the movement was directed, since neither Lees (1943*b*) nor the present writer (Falconer, 1945) found any evidence of a gravitational response in burrowing wireworms, an experiment was nevertheless carried out to test the point. The technique differed from that of the foregoing experiments. Four glass troughs, measuring 8 in. × 2 in. × 2 in. and covered by glass lids, were obtained. Into two of the troughs vertical glass partitions, 1½ in. high, were fixed at a distance of 1½ in. from one end, dividing each into a small and a large compartment (see Fig. 2). They were filled with moist sand to the level of the top of the partition, and the wireworms

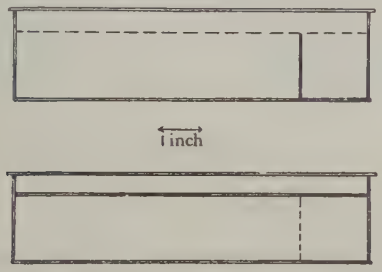

Fig. 2. Troughs used to compare migrations over the surface (upper figure) and through the sand (lower figure).

were placed initially in the small compartment. There was thus a surface area of 1½ in. × 2 in. (= 3 sq. in.) through which the wireworms could migrate. The much larger area of the originally unoccupied compartment allowed a relatively large proportion of those actually emerging to be recorded. The other two troughs were not partitioned in this way. They were filled with sand to the same depth of 1½ in. and the wireworms were placed in the same relative position as in the partitioned troughs. The sand was then covered with a glass plate resting on the surface, so that no surface migration could take place (see Fig. 2). At the end of each experiment, before the animals were counted, the sand was divided accurately at a distance of 1½ in. from the end at which the wireworms were placed: at a position, that is, corresponding to the partition in the other troughs. Now, the plane of this division represented an area of 1½ in. × 2 in. (= 3 sq. in.) of sand, through which the wireworms could migrate. Thus the numbers migrating over the surface (in the first pair of troughs) and through the sand (in the second pair of troughs) were directly comparable, and if emergence were a random movement, these numbers would show no difference.

Ten wireworms were used in each trough, and the experiments lasted 2 days each, the wireworms being changed from one to the other type of trough at each experiment. No food was given. These experiments were done at a constant temperature of 17·5° C. Saturation of the atmosphere above the sand was ensured by covering the troughs with damp cotton-wool.

For comparison of the numbers migrating over the surface and through the sand, there were fourteen observations of each. The results were as follows:

	Mean		Standard error
No. migrating over surface	0·9	±	0·401
No. migrating through sand	1·8	±	0·344

Fewer animals migrated over the surface than horizontally through the sand, and therefore the wireworms' coming to the surface cannot be regarded as a directed movement. In fact, when the observations were tested for conformity with the hypothesis that an equal number migrated over the surface and through the sand, a value of 10·5 was obtained for χ^2 (with five degrees of freedom). The difference was, therefore, significant at the 10% level, and the experiment suggests that some of the animals, when they encountered the surface, did not emerge, but retreated into the sand again.

Factors influencing the point of re-entry

It was shown above that the wireworms' emergence was a random movement, and that it was much reduced by the presence of food in the sand. It is, therefore, possible that the surface movement was of a food-seeking nature; but its success as such would clearly depend on the wireworms' ability to locate food by means of the condition of the surface over which they crawled. A series of experiments was accordingly undertaken with the object of determining the influence of various factors on the point of re-entry of wireworms crawling on the surface.

The technique was that of the first experiments, but in this case it was necessary to provide alternative conditions in the compartments for re-entry. The wireworms were accordingly placed in compartments 2 and 5 (twenty in each), and of the remaining compartments (which could be entered only by surface migration) nos. 1 and 6 presented the condition under investigation, while nos. 3 and 4 presented the alternative. In order to reduce as far as possible the likelihood of a wireworm leaving the compartment which it first entered, food (soaked, dead wheat grains) was placed below the surface in all four 'new' compartments, but not in the two original ones (nos. 2 and 5).

Sprouting wheat. The condition tested first was the presence of sprouting wheat. Living wheat was sown in compartments 1 and 6, and after it had sprouted to a height of about 2 in. above the surface the experiment was performed. Two experiments were carried out, in the first of which the animals

were left for 5 days before examination, while in the second they were left for 1 day. The numbers found in the different compartments were as follows:

Experiment no. ...	1	2
In compartments with sprouting wheat	19	24
In compartments without sprouting wheat	1	2

Re-entry was thus very much more frequent in the compartments with sprouting wheat.

The influence of sprouting wheat clearly required further analysis, for it might have been due to several factors, namely chemical factors, the presence of solid objects sticking out of the sand, or the texture of the surface of the sand. In connexion with the latter factor it should be pointed out that wireworms were known to burrow more frequently into rough surfaces than smooth (Subklew, 1935), and for this reason efforts were made in the above experiments to equalize the texture of the surface in the compartments with sprouting wheat and in those without. It is probable, however, that this condition was not fully realized.

Dissolved food substances. Nine experiments with food substances in solution were carried out. For this purpose an extract was made by cutting up a piece of raw potato in a little water and filtering.

Twenty-five drops of the solution were placed on the surface of the sand in compartments 1 and 6, and an equal quantity of water in compartments 3 and 4. Each experiment lasted 1 day, and the final positions of the wireworms were as follows:

Exp. no....	1	2	3	4	5	6	7	8	9	Totals
With food substance	18	0	11	5	3	19	3	0	3	62
Without food substance	1	0	4	7	23	6	1	2	3	47

Though there was a slight excess of animals entering the compartments with the food substance, the results are inconclusive on account of their irregularity. It does not seem probable that the presence of dissolved food substances exerted much influence on the point of re-entry.

Glass rods in sand. Twenty-five glass rods, 3 mm. in diameter, were placed vertically in each of compartments 1 and 6. The rods were tapered at the lower end and were placed in position with care, in order to avoid the formation of depressions in the sand at their bases. Only two wireworms entered the new compartments, both into one without rods; but the presence of holes in the surface of the sand in the original compartments showed that many wireworms had emerged, but had re-entered the original compartments. It appears unlikely, therefore, that the presence of solid objects sticking out of the surface of the sand influenced the wireworms' point of re-entry.

Holes in surface. Twenty-five holes about 1 cm. deep and 0·4 cm. in diameter were made with the point of a pencil in each of compartments 1 and 6. Thirteen wireworms entered the compartments with holes and none those without. Though perhaps in itself not fully conclusive, the result of this experiment in conjunction with that of the last suggests strongly that wireworms re-entered the sand more readily where there were holes than where the surface was smooth.

Texture of surface. The influence of the texture of the surface was investigated by covering the surface of compartments 1 and 6 with coarse sand (particles 2–4 mm. in diameter). Only seven animals entered new compartments, four in the coarse and three in the fine sand. This negative result cannot be regarded as contrary to that of Subklew (1935), since he observed the attempts of wireworms to burrow into a surface which they could not enter, while in the present case the success of the burrowing, and not the attempt, was observed.

The conclusion to be drawn from the above experiments on the influence of various factors on the point of re-entry appears to be that the chief factor was the ease with which entry could be made, rather than any particular stimulus resulting from the condition of the surface.

Effect of light on surface migration

It was shown in an experiment described above that wireworms came to the surface even under strong illumination. It was of interest, therefore, to determine whether the point of re-entry was influenced by the intensity or the direction of light, or whether the conditions which led to spontaneous emergence resulted also in an inhibition of the normal reaction to light. The following experiments were therefore carried out.

Effect of intensity of light on point of re-entry. The surface of the sand was illuminated from above by a 60 watt lamp placed 42 in. above the surface. Compartments 3 and 4 were darkened by means of black paper fixed to the lid of the dish, and twenty wireworms were placed in each of compartments 2 and 5. After an interval of 4 days the animals in compartments 1 and 6 and in 3 and 4 were counted and then returned to compartments 2 and 5, which were otherwise undisturbed. This was repeated five times at intervals of 2 days, and the numbers which entered the dark and light compartments were as follows:

Exp. no....	1	2	3	4	5	Totals
No. in dark	15	13	5	15	8	56
No. in light	17	4	8	13	17	59

The result shows conclusively that the intensity of illumination had no influence on the point of re-entry.

Effect of direction of light on direction of surface migration. The wireworms were placed in compartments 2 and 5, and a light (60-watt lamp) was placed at a distance of 12 in. and at a height of 6 in. above the surface of the sand, in such a way that migration to compartments 1 and 4 was toward the light and to

compartments 3 and 6 away from the light. Three experiments were carried out and the results were as follows:

Exp. no. ...	1	2	3	Totals
Toward light	5	0	1	6
Away from light	16	11	24	51

It is clear, therefore, that the direction of the light strongly influenced the direction of movement of the wireworms on the surface of the sand.

Time spent on surface

Observations of the number of wireworms which were on the surface at a particular moment were made from time to time during the course of the work. The observations will not be described in detail, but it will merely be stated that they led to the following tentative conclusion. When no food was present in any of the compartments, the average proportion of time spent by each animal on the surface was about 1/12. Under these conditions there was nothing to prevent several visits being made to the surface by each animal, and so it is impossible to state the average duration of one visit. On the other hand, when food was present in the new compartments and conditions were most favourable for re-entry into the new compartments, the average proportion of time spent on the surface was about 1/48. Under these conditions it is probable that each animal made only one visit to the surface, and if this were the case the average duration of each visit must have been about ½ hr.

Now, the average speed at which wireworms move over a flat surface is about 8 cm. per min. at 10°C. and 18 cm. per min. at 20°C. (Falconer, 1945). This means that, under the conditions of the experiment, the average distance which the wireworms would travel over the surface before re-entering would be, very approximately, 2½ m. at 10°C. and 5½ m. at 20°C.

DISCUSSION

Animals cannot be expected to behave in the laboratory exactly as they do under natural conditions, and in the absence of confirmatory field experiments, it would be rash to apply the conclusions drawn from the foregoing experiments to wireworms in their normal environment. Yet nothing in the laboratory experiments suggested that the behaviour exhibited by the wireworms would be limited to the laboratory, and if it were found to take place also under natural conditions of food shortage, the fact would clearly be important in connexion with the migration of wireworms out of regions where food was scarce. The possible influence of various factors on the movement of the wireworms while on the surface are, therefore, worthy of consideration.

In the first place, if surface migration were to be effective in taking the animals out of a region of food shortage, their movement would have to take place in a reasonably straight line. In the absence of any means of orientation, movement would be random in direction, and the effective distance travelled would be small. The wireworms were found, however, to react to directional light while crawling on the surface, and this response could clearly provide a means of orientation which would cause the animals to follow a more or less straight path. On the other hand, surface migrations would take place only when the humidity of the air was very high, and would therefore be expected to occur principally at night, when there would be little light. Now, experiments (described in an appendix to this paper) have shown that wireworms are extremely sensitive to light, and are able to react to the light of the moon, and even to light of much lower intensity. It is probable, therefore, that even at night there would often be sufficient light to enable the wireworms to travel over the surface in a more or less straight path. The definite reactions and extreme sensitivity of wireworms to light are not easy to understand, and the above may be tentatively suggested as a possible function.

In the second place, the condition of the surface of the soil would influence the distance travelled by the wireworms. Holes or crevices or a loose texture would favour early re-entry, whereas a smooth, hard surface would prolong the period spent on the surface. It is impossible to say how far the wireworms might travel over the surface, but if conditions resembled those of the laboratory experiments, they might cover a distance of some metres during the course of a single period of emergence.

SUMMARY

Wireworms, when kept in moist sand without food, moved about at random in the sand, and this movement sometimes brought them to the surface. When this happened, their responses to light and to lack of contact did not prevent them from emerging on to the surface and crawling about there for a considerable period of time. On the other hand, their response to the humidity of the air prevented their emergence into air of 85% (and probably much higher) relative humidity. Though the emergence was conditional upon lack of food, the movement of the wireworms on the surface is hardly to be regarded as of a 'food-seeking' nature, for they appeared to have no direct means of locating their food: the point of re-entry was determined principally by the ease with which the animals could burrow beneath the surface.

Wireworms were found to be extremely sensitive to light of all wave-lengths including the red, the threshold intensity to which they responded being below that required for their observation.

Appendix

SENSITIVITY OF WIREWORMS TO LIGHT

Wireworms have been shown by Subklew (1935) to have a strong negative reaction to light, a fact that may easily

be verified by placing a number of wireworms on a table in front of a window. There appears, however, to be no information in the literature about the sensitivity of wireworms to light, and for this reason some experiments were performed with the object of determining approximately the threshold of sensitivity to light of different colours.

The experiments were made with large specimens of *A. lineatus* or *A. obscurus*. The movements of the animals on a large sheet of damp paper, when illuminated horizontally by light of various colours and intensities, were observed. It was found that the wireworms were extremely sensitive to light of all colours, and, while there was considerable variation, some individuals reacted when the light was so weak that the animals could not be seen. For this reason determination of the actual threshold was not achieved. Definite reactions to light of all colours were obtained from some individuals at the lowest intensities tested, of which approximate values in metre-candles are given in Table 2.

Table 2

Colour	Wave-lengths transmitted, $\mu\mu$		Intensity in metre-candles (approx.)
Red	All above	620	0·3
Light red	All above	560	0·1
Green	Maximum at	540	0·01
Blue-green	Maximum at	510	0·005
Blue	Maximum at	460	0·005
Violet	Maximum about	430	0·05

Records of the movement of one individual are shown in Fig. 3. The records were made by noting the original and final positions of the animal when allowed to crawl for 1 or 2 min., and the lines show the approximate paths followed. The animal was illuminated by red light (i.e. wave-lengths above 620 $\mu\mu$) and six trials were made at each of four intensities. The animal was started with its head at the point marked with a circle, and was orientated transversely to the direction of the light, which is shown by arrows. Tracks shown by full lines were made when the animal was illuminated from the left side, and those by dotted lines when illuminated from the right side. It is clear that the threshold of sensitivity to red light of this particular individual lay between about 0·05 and 0·1 metre-candles.

A further experiment was carried out in order to see whether this high sensitivity would enable wireworms to make use of moonlight as a means of orientation. The experiment was performed when the moon was 2 days 16 hr. past the full, and at an elevation of 20°. Fifteen wireworms were placed in turn with random orientation near the centre of a large sheet of moist paper, and their movements were followed with a pen and recorded on the paper. The tracks, arranged so that each starts from the same point, are shown in Fig. 4. The conclusion is beyond doubt: fourteen out of fifteen wireworms were clearly able to orientate, and moved away from the source of light.

It is hard to see what functions the definite reaction and high degree of sensitivity to light might play in the life of an animal which lives mainly in the soil, but the results obtained may be of some practical importance in showing that the greatest care must be taken in the technique of experiments dealing with the behaviour of wireworms, if there is any possibility of light influencing the animals' reactions.

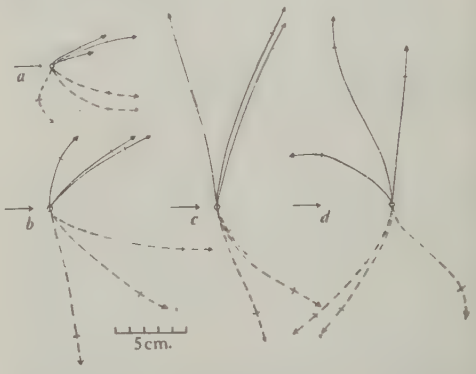

Fig. 3. Approximate paths followed by a wireworm in a horizontal beam of red light of different intensities. Approximate intensities were: (*a*) 0·46 metre-candles, (*b*) 0·17 metre-candles, (*c*) 0·09 metre-candles; (*d*) 0·05 metre-candles. Further explanation in text.

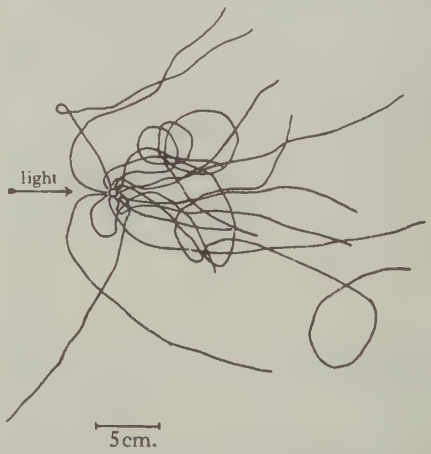

Fig. 4. Record of paths followed by fifteen wireworms when illuminated by the moon at 20° elevation. All tracks start at the circle.

REFERENCES

FALCONER, D. S. (1945). *J. Exp. Biol.* **21**, 17.
GUÉNIAT, E. (1937). *Mitt. schweiz. ent. Ges.* **16**, 167.
LEES, A. D. (1943*a*). *J. Exp. Biol.* **20**, 43.
LEES, A. D. (1943*b*). *J. Exp. Biol.* **20**, 54.
SUBKLEW, W. (1934). *Z. angew. Ent.* **21**, 96.
SUBKLEW, W. (1935). *Z. vergl. Physiol.* **21**, 157.

OXYGEN CONSUMPTION OF PREPUPAE OF *DROSOPHILA MELANOGASTER* MEIGEN, IN RELATION TO THE SURFACE AREA OF THE PUPARIUM

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(With Four Text-figures)

INTRODUCTION

Measurement of the oxygen consumption of early prepupae of *Drosophila melanogaster* Meigen showed that small prepupae consumed more oxygen, per mg. wet weight, than animals of larger size (Ellenby, 1938). It subsequently proved possible to measure puparial surface area, and in the present paper the data dealing with rate of oxygen uptake are analysed in relation to the surface area measurements. (See also Ellenby, 1937.)

MATERIALS AND METHODS

(*a*) *Oxygen consumption*

The techniques employed have already been described in full (Ellenby, 1938); only the chief points will therefore be indicated.

The flies used belonged to the two lines, *vestigial* and *wild-type*, the latter obtained after repeatedly backcrossing a *wild-type* stock to the *vestigial* line. The male and female of each type were examined, and, in addition, the male prepupae of a *vestigial-wild-type* cross. All animals were raised at 24·5 ± 0·2° C. on the maize-meal culture medium used in the laboratory at University College, London. Prepupae were 0·5 ± 0·5 hr. old when obtained from the cultures. Their surfaces were sterilized by immersion in 90% alcohol, and the sexes separated. The oxygen consumption of one of these groups, usually comprising not less than ten individuals, was determined at 15 min. intervals at a temperature of 26·85 ± 0·05° C., and the prepupae then rapidly weighed on an automatic balance. Counting the time taken to carry out the preliminary work of separating the sexes, preparing the respirometer, etc., the whole experimental period was included in the first 5 hr. of the prepupal period; the latter is of 11 hr. duration at 25° C. (Bliss, 1926). The entire puparial period, i.e. the interval between the cessation of larval movements and the emergence of the imago and therefore including the prepupal period, is eight or nine times the duration of the latter period.

(*b*) *Surface area*

The decision to investigate the relation of surface area and metabolic rate was only taken after the respiration experiments had been completed; it was impossible, therefore, to use prepupae of known oxygen uptake for the surface-area measurements. Accordingly, other animals were used which belonged to the backcrossed *wild-type* stock already mentioned, the prepupae being collected and treated in exactly the same way as that outlined above; the weight data from both sets of determinations are therefore strictly comparable. Special efforts were made to obtain prepupae from the entire range of body sizes covered in the respiration experiments.

The size of the prepupae obtained from a culture can easily be altered by varying the concentration of eggs (Ellenby, 1938). Accordingly, young females were mated for 4 days, and then allowed to lay eggs for 24 hr. in previously yeasted culture bottles. Five sets of bottles were used, and the number of females per bottle was varied from two to six for the different sets. The flies were transferred to a similar set of bottles after 24 hr. and they were only used for egg production for five consecutive 24 hr. periods. Prepupae were collected as before, cleaned, separated into male and female groups, and then placed in the respirometer for 3 hr. at 26·8° C. in the usual way before their weight was determined. Although prepupae varying in mean wet weight from 0·87 to 1·565 mg. were obtained by this method, it must be emphasized that each member of a group from a particular set of cultures would tend to be more or less of the same size as other members of the same group.

After weighing, the prepupae were fastened in a row to a microscope slide by a thin film of commercial gum, all prepupae pointing in the same direction The slide was then placed in a corked tube with a little water until the flies emerged. This procedure had formed part of the respiration experimental routine as it was necessary to see whether the flies examined were normal in all respects; it was readily

adapted to form part of a method for the determination of the surface area which, later, was found to be essentially similar to that used by Simanton (1933) for the measurement of the surface area of the bean aphid and the German cockroach.

In *Drosophila* the imago emerges from a region of the puparium, called the operculum by Strasburger (1935), which is modified very early in puparial life (Robertson, 1936). The puparium is therefore left behind on the slide, when the fly has emerged, always split in the same region. If another slide is placed on top of the slide to which the empty puparia are attached so that it first touches their posterior ends, they are flattened, in most cases, without breaking. Great pressure is not necessary in order to make the puparia as flat as possible, for it was shown that

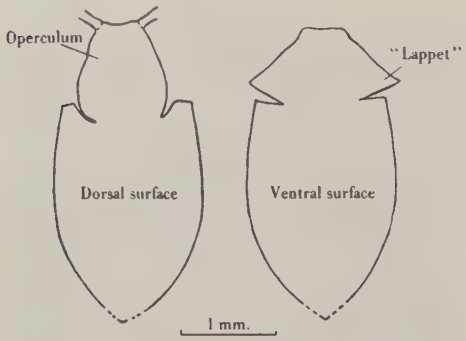

Fig. 1. Outline of image of flattened puparium.

increase in the pressure exerted made no difference detectable with the high power of a microscope. The process should be carried out immediately after the slide bearing the puparia is withdrawn from the tube, for the gum rapidly hardens on exposure to air and this prevents the empty puparia from flattening freely.

The images of the puparia were projected on to smooth paper at a linear magnification of × 30, and dorsal and ventral surfaces separately outlined (Fig. 1). Areas were measured with a planimeter.

The puparium is slightly distorted, particularly at the posterior end which is somewhat wrinkled. When outlining the projected image, the general outline was continued posteriorly and anteriorly so as to exclude the spiracles, as indicated in Fig. 1 by the dotted lines; these regions were also omitted in the measurements of the surface area.

Fig. 1 shows that the operculum splits away from the rest of the puparium along its anterior and lateral margins, but remains attached and hinged at its posterior end. When the puparium is flattened, the edges previously united to the lateral margins of the operculum fold outwards to give the two 'lappets' shown in the figure. Interpretation of the projected images was considerably facilitated by this occurrence. There is little doubt that the presence of the operculum is responsible for the singularly little distortion which the flattened puparium exhibits.

Flattened puparia have been kept for over a year with no preservative whatsoever, the pairs of slides remaining firmly attached. There is a slight tendency for mould to grow on the gum, but this scarcely affects the clarity of the image. Before projecting, a little water should be run between the slides, and then, resting them on a flat surface, they should be tapped vigorously with a piece of soft wood such as the tip of a brush handle. This expels the air bubbles imprisoned in the puparium and greatly improves the clarity of the image.

It might be of some interest to mention that a rough attempt was made to measure the thickness of the flattened puparia, as it was considered that the validity of the method would be affected if the puparia were elliptical, rather than flat, in cross-section. Accordingly the thickness of the two slides was measured, (*a*) when adhering together, with the puparia between, (*b*) when separated and individually, and (*c*) when pressed together as tightly as possible, but with no puparia between and not adhering. Using a micrometer screw gauge, accurate to 0·001 in., it was found that the thickness of the flattened puparia was less than the limits of accuracy of the gauge.

RESULTS

(*a*) *Oxygen consumption and body weight*

The rate curve of oxygen consumption for the puparial period of insects shows three main stages. An initial stage of high but falling respiratory rate is followed by a stage during which it is rather low but fairly steady; finally there is a stage of increasing rate. It has been shown that the curve for *Drosophila* conforms to this pattern (Bodine & Orr, 1925; Clare, 1925; Orr, 1925; Poulson, 1935; Dobzhansky & Poulson, 1935; Wolsky, 1938). The period dealt with in this work lies, therefore, in the region of rapidly falling oxygen consumption, and it has already been shown that the rate at the end of the experimental period, i.e. 5 hr. after puparium formation, is about 35 % lower than the rate 3 hr. earlier (Ellenby, 1938). During this period the curves for males, females, and the various genotypes are of the same shape, so that it is permissible to average, for each experiment, the rate of oxygen consumption per hour of the experimental period. These mean values, based on over 700 animals in groups, have been plotted against the corresponding mean body weights in Fig. 2, different symbols being used for the different types of prepupae.

The progressive decrease in rate of oxygen uptake is well shown. There is a difference of 0·853 mg. between the two extremes of the weight range, and

this difference of body weight corresponds to a difference in rate of oxygen uptake of 0·0878 mm.³ per mg. per hr. The smallest animals thus have, per mg., an oxygen uptake 50% greater than that of prepupae almost exactly twice their wet weight. It will be remembered that these large differences are due neither to the sex nor the genotype of the prepupae, and that they are, in fact, due to differences in body size; animals of similar size, but belonging to different genotypes, showed no such differences in their respiration (Ellenby, 1938). Clearly, if the factor of body size is not controlled, the most misleading results could be obtained; there is little doubt that it is responsible for some of the variation in

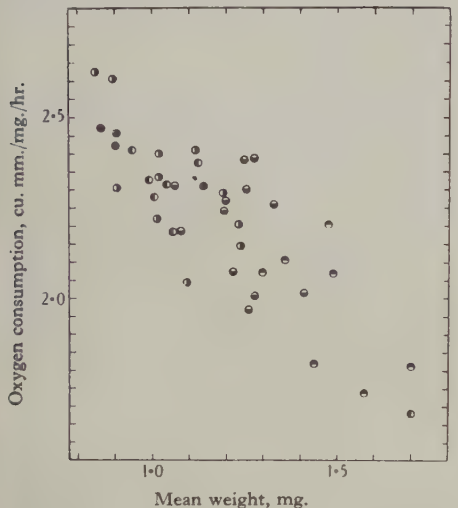

Fig. 2. Oxygen consumption, per mg., in relation to body weight. *Wild-type* ♂ ◐, *Wild-type* ♀ ◑, *vestigial* ♂ ◒, *vestigial* ♀ ◓, heterozygote, ♂ ●.

oxygen consumption, per mg. body weight, reported by other workers on *Drosophila*. Furthermore, different groups of animals, even of the same mean weight, would almost certainly have different size compositions; as the oxygen consumption is not directly proportional to the weight, this lack of homogeneity in groups would manifest itself by variations in the results when calculated on the basis of mean weights. Some of the scatter in Fig. 2 will be due to this cause.

(*b*) *Surface area and body weight*

The surface area of 173 puparia in 22 groups ranging in mean weight from 0·87 to 1·565 mg. was measured. For comparison with Fig. 2 which showed the relationship between oxygen uptake per mg. and mean wet weight, Fig. 3 was constructed; in this, mean surface area, per mg., has been plotted against the mean wet weight for each group. The points lie very evenly about a straight line, the slope of which expresses the rate at which surface area per mg. decreases with increasing mean wet weight. These two variables should, theoretically, show a curvilinear relationship; in fact, consideration of the position of the origin in relation to the graph suggests that the straight line forms a small part of a large curve. At all events, it is clear from the figure that the calculated linear regression line fits the data with considerable fidelity, the correlation coefficient being 0·92, which is very high for data of this sort.

In studies of the relationship of surface area and metabolism, it is obviously desirable to know the

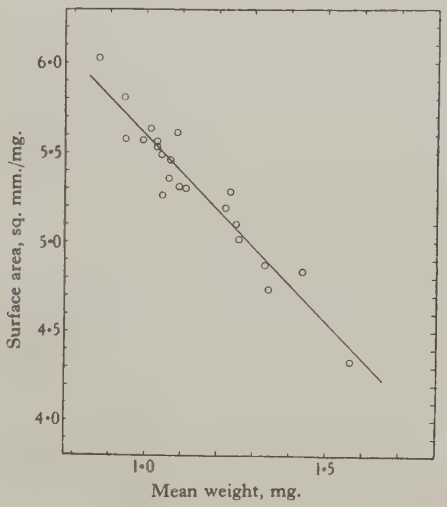

Fig. 3. Surface area, per mg., and body weight.

surface area of the actual animals used in the metabolism experiments. This is rarely possible, however, as most methods for determining surface area are far too laborious. In most cases it is also usually necessary to kill the animal before its surface area can be determined, and this is sometimes undesirable. Neither of these problems arises in the case of *Drosophila*, or perhaps in the case of any of the Holometabolous insects. Unfortunately, as already mentioned, the measurements of oxygen consumption were completed, in the present case, before it was decided to investigate its relationship with puparial surface area. In such cases the surface area is usually estimated from the weight, using a formula of the general type, surface area = constant × weight^n, where n generally approximates to $\frac{2}{3}$. This was found to be unsatisfactory; in any case, the surface area can be estimated directly from the regression equation.

(c) Surface area and oxygen consumption

The regression line of Fig. 3 was fitted to the data from the regression equation

$$E_1 = 5{\cdot}3118 - 2{\cdot}1099\,(X_1 - 1{\cdot}1342),$$

where E_1 = the estimated surface area, per mg., of prepupae of mean wet weight X_1. This equation, calculated from the data of Fig. 3 (Snedecor, 1938), provides a ready means for estimating the surface area, per mg., of prepupae of known mean wet weight; as the standard error of estimate = ±0·117,

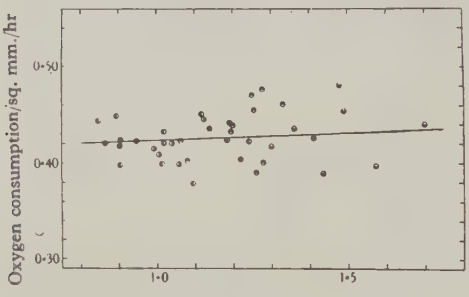

Fig. 4. Oxygen consumption, per sq. mm. of body surface, in relation to body weight. *Wild-type* ♂ ◐, *wild-type* ♀ ◑, *vestigial* ♂ ◒, *vestigial* ♀ ◓, heterozygote ♂ ●. The line fitting these data does not differ significantly from a line parallel to the weight axis.

values for 'size groups' rather than for different types of animals, for comparison of different types of prepupae of the same size showed that there were no differences in oxygen consumption per mg. wet weight. The success of the transformation to a surface-area basis is clear; it is brought out sharply by a comparison of the magnitudes of the standard deviation (S.D.) in the different cases. Proceeding down column 3 of Table 1, the S.D. is 7·7, 11, 15, 9 and 3·5% of the mean values, respectively; the corresponding values for column 4 are 5·4, 2·8, 7, 6·8 and 2·6%. It is interesting to note that even in the case of the most homogeneous group, the heterozygous males, where the values for oxygen consumption per mg. are in close agreement (S.D. = 3·5% of mean value), the agreement in the values for oxygen consumption per sq. mm. are even closer (S.D. = 2·6% of mean value).

Inspection of the mean values for oxygen consumption per sq. mm. suggests that there are no differences between the various groups; this is confirmed by statistical analysis, for an analysis of variance showed that the mean squares 'between groups' and 'within groups' were almost equal ($F = 1{\cdot}07$, $p > 0{\cdot}20$). Pooling the data, the mean value for oxygen consumption per sq. mm. per hr. is 0·4262 ± 0·225; the corresponding value for oxygen consumption per mg. wet weight per hr. is 2·221 ± 0·265. Calculation of oxygen consumption on a surface area basis thus reduces the S.D. from 12% of the mean to 5·3%; considerably more than half the variation in oxygen consumption per mg. shown in

Table 1

Type	Mean wet weight mg.	Mean O_2 uptake per mg. wet weight per hr. and S.D.	Mean O_2 uptake per sq.mm. per hr. and S.D.	Regression coefficient, O_2 uptake per sq.mm. per hr. and mean wet weight
Wild-type, male	1·048	2·327 ± 0·179	0·4235 ± 0·0228	+0·0093
Wild-type, female	1·124	2·225 ± 0·248	0·4169 ± 0·0162	−0·0014
Vestigial, male	1·235	2·201 ± 0·334	0·4307 ± 0·0303	−0·0070
Vestigial, female	1·476	1·976 ± 0·180	0·4328 ± 0·0293	+0·0071
Heterozygote, male	0·954	2·415 ± 0·085	0·4245 ± 0·0110	+0·0650
Pooled data	1·1799	2·221 ± 0·265	0·4262 ± 0·0225	+0·0141

or only 2·2% of the mean, this can be done with considerable accuracy. Accordingly, the estimated surface area, per mg., for the animals of known oxygen consumption of Fig. 2 was calculated, and thus the oxygen consumption per sq. mm. of body surface. These values are plotted against mean wet weight in Fig. 4, while in Table 1 the mean values for the various types of prepupae examined are tabulated. The close agreement in the values of oxygen consumption per sq. mm. for the different types will be noted; for comparison, the mean values for oxygen consumption per mg. wet weight have also been tabulated, although these latter values are in reality Fig. 2 is therefore due to the fact that prepupae of different wet weight have different rates of oxygen consumption per mg.; and part of the remaining variation attributable to experimental error, includes, as already mentioned, a 'reflexion' of the size factor in the error due to examining groups of animals instead of individuals.

Comparison of S.D.'s, however, although useful, does the data scant justice, for each value of oxygen uptake per mg. wet weight is perfectly valid as an indication of oxygen consumption of prepupae of a particular size. That is to say, the data of oxygen consumption per mg. constitute, not one population

of results, but a series of such populations for animals at different levels of body size. It is more legitimate, in a rough comparison of the two standards wet weight and surface area, to compare the differences between the two extremes of the size range, for both sets of data. It will be recalled (p. 41) that the level of oxygen consumption per mg. wet weight was 50% greater for the smallest animals than for the largest; for these same animals, the values of oxygen consumption per sq. mm. of body surface differ by only 2%. In fact, the regression line in Fig. 4 shows that in the latter series of values there is no significant variation with increasing body weight whatsoever.

The values of the regression coefficients for the different types of prepupae are shown in column 5 of Table 1. Except for the heterozygote, which is based on only four experiments, the values are very close to zero. The regression equation calculated for the pooled data is

$$E_s = 0{\cdot}4262 + 0{\cdot}0141\ (X_s - 1{\cdot}1799),$$

where E_s is the estimated oxygen consumption per sq. mm. per hr. of prepupae of mean wet weight X_s mg. and the S.E. of estimate $= \pm 0{\cdot}0226$. Fig. 3 shows how closely the regression line approaches the horizontal; starting from the mean wet weight of 1·1799 mg., an increase in wet weight of 0·5 mg., which is rather more than the experimental range from the mean, the oxygen consumption per sq. mm. of puparial surface increases by only 0·007 mm.^{3}, or by roughly $1\frac{1}{2}$%; as the S.E. of regression is $\pm 0{\cdot}0164$, the regression coefficient does not differ significantly from zero ($p = 0{\cdot}40$, a very high value indeed). It can therefore be said that oxygen uptake, per unit of surface area, is constant throughout the range of mean wet weight.

DISCUSSION

The results clearly indicate that the oxygen consumption of early prepupae of *Drosophila melanogaster* is proportional to the surface area of the puparium. Under ordinary cultural conditions (Ellenby, 1938), male prepupae, for example, were found to consume more oxygen, per mg. wet weight, than female prepupae; these differences were due to the fact that, in these conditions, female prepupae tend to be larger than male. If female and male prepupae of similar size are compared, they do not differ at all in their oxygen consumption. Had the rate of oxygen consumption been calculated 'per unit of surface area', the original differences would never have been recorded, even for males and females differing widely in body size. It is clear, therefore, that the latter method of expressing results does not mask genuine differences between oganisms; it merely eliminates those differences which are due to body size. 'Surface area', then, provides a standard which, in so far as it eliminates the size factor, is infinitely more useful as a standard than wet weight; using it, comparison may be made of the oxygen consumption of early prepupae of *D. melanogaster* differing in size, genotype, and sex.

The method described for measuring surface area is sufficiently simple to be of practical use; moreover, the equation relating wet weight and surface area makes it possible to estimate the surface area of animals of known wet weight with considerable accuracy.

Although 'surface area' and '*wet* weight' have been compared as standards, there is no reason for thinking that '*dry* weight' would be more satisfactory. Determinations of the dry weight at 102°C, of over 2200 prepuae in 45 groups differing in sex, genotype, and body size, showed that the wet weight/dry weight ratio was $3{\cdot}26 \pm 0{\cdot}106$; the ratio showed no apparent change with increasing wet weight. Wolsky (1938) reported the ratio to be about 2·2; but this was for very much older pupae and indicates the enormous water loss during the puparial period.

The utility of 'surface area' as a unit for comparing the metabolic rates of animals differing in species and in size is only one aspect of the so-called 'surface-area law': the second concerns itself with the cause of the variation, with body size, of metabolic rate per unit of weight, or with the nature of the relationship of metabolism and surface area. Both these aspects have been extensively reviewed mainly in relation to vertebrates by Benedict (1938). Much less is known in the case of invertebrates, although there are examples from most phyla where the metabolic rate has been shown to vary with the size of the animal, and some cases in which it has been suggested that it varied with the surface area; most of these examples have recently been cited (Wingfield, 1939; Whitney, 1942). Much of the work which they mention, however, is open to serious criticism on three grounds.

(1) In most cases, 'basal metabolism' is not measured, as the animals are allowed to move about freely during the course of the determinations; the efficiency of movement, and therefore its energy relations, will vary with the size of the animal.

(2) In the case of aquatic animals, with which much of the work cited is concerned, osmotic and ionic regulation may play a considerable part in influencing metabolic differences in relation to body size. It is true that this forms quite a valid portion of 'basal metabolism', but as different aquatic animals may have different relationships of this sort, comparisons between them may be unwise.

(3) Comparison between animals of different size is usually complicated by the fact that 'body size' also coincides with 'age'.

These criticisms do not apply to the results described in the present paper, the only variables being those connected directly with body size. Yet although the metabolic rate has been shown to be undoubtedly proportional to the surface area, it would be rash indeed to consider a period of great embryo-

logical activity, such as the one examined in the present work, to be one of 'basal metabolism', even though the animals are superficially motionless, and to compare, quantitatively, the results presented in the present paper with those for other animals. With surface area as a standard, however, it should be instructive to compare the metabolic rates of other insects, during corresponding prepupal and pupal periods, particularly the other species of *Drosophila*.

Although it has been demonstrated that the rate of oxygen uptake is proportional to the surface area, this is vastly different from the demonstration of a causal relationship between the two. Indeed, the true surface area of the puparium was not measured, but only its apparent surface area; it is not generally appreciated how greatly these two may differ. For example, Bowden & Rideal (1928), Bowden & O'Connor (1930), and Vernon (1926, 1935) have all shown that the true surface of a polished metal plate may be between two and ten times as great as its apparent surface. At best, therefore, the measurements made in the present work were of a surface which was directly proportional to the true surface area: and there may be other systems which also bear this relation to the true surface area. There is little reason to suppose that the extent of puparial surface functions as a limiting factor in metabolism, for, if such were the case, large pupae would take longer to develop than small ones and, as far as I am aware, there is no evidence of this. Moreover, according to Robertson (1936), the supply of air via the anterior spiracles is uninterrupted throughout the puparial period, and, as the cuticle is almost certainly permeable to gases, as Fraenkel & Herford (1938) have shown for the larva of the larger *Lucilia sericata*, an animal of 1 mm. diameter or less could, in any case, obtain sufficient oxygen by diffusion alone.

Benedict & Talbot (1921) suggest that it is best to regard the surface-area law as illustrating some general morphological law of growth. This seems to be the most satisfactory position to take up at the moment, for the law can be regarded as a comprehensive physiological example of the law of allelometric growth (Huxley, 1932) which has been demonstrated so frequently for morphological data. Teissier (1929, 1931) and Needham (1934), among others, have shown the application of the law to biochemical relationships; and the morphological and biochemical can be regarded as having their physiological reflexion in the surface law.

It needs to be emphasized, however, that it would be unwise at this stage to generalize from one type of animal to another, and, like Bodine (1921), Bodenheimer (1929), and Butler & Innes (1935), to suggest, that because the metabolic rate of both vertebrates and some insects is proportional to the area of their body surface, that the reason for this is necessarily the same. Discussion of one theoretical aspect of the law will make this more clear.

It has been suggested on numerous occasions that the surface area law was due to the fact that metabolic rate was controlled by the extent of cell surface, and that the latter varied at the same rate as the superficial surface area of the body. Both Rubner (1913), and Pfaundler (1921) have shown the fallacy in this, for, clearly, this theory, so acceptable on some grounds, could only be tenable if the volume of the individual cells increased at the same rate as the volume of the whole body. In fact, it is well known that, with the exception of nerve cells, the size of most cells remains more or less constant as animals grow, while similar types of cells of animals of widely different size are more or less of the same size. Yet it is certain that this is not the case, at least for some insects, so that the cell surface theory may be valid for them, if only intraspecifically. Both Trager (1935), working with *Lucilia sericata* and *Bombyx mori*, and Abercrombie (1936), with *Popillia japonica*, have made very careful measurements of the actual size of cells of a variety of different tissues in the different larval stages. In the case of *Lucilia sericata*, Trager found that the entire growth of the larva from egg to pupa is accounted for by increase in the size of cells, while Abercrombie found that the same is largely true of the larva of *Popillia japonica*. In addition, Loewenthal (1923) showed that the differences in body size of large and small pupae of *Calliphora erythrocephala* were due to differences in the size of the cells, while Alpatov (1930) showed with *Drosophila melanogaster* that, in differently fed flies, increase in cell size could account for most of the increase in body size. Trager's work with *Bombyx mori* (1935) showed, however, that these results do not apply to insects as a whole: whereas cells of certain tissues increased in size during an instar at the same rate as body size, some cells remained the same size, while others increased in size, but not at the same rate as body size. Moreover, cells of different tissues differed in their growth behaviour from larval stage to stage. For example, the hypodermal cells increased in length during the first instar, remained constant in length during the second and third instars, and increased again in the fourth. It is interesting to compare this with the observations of Butler & Innes (1935) that the oxygen consumption of locusts, per unit of surface area, decreased from the first to the third instar, and then increased from the fourth instar onwards; clearly, if a cell-surface theory has any validity, comparison of the metabolism of animals exhibiting these different methods of growth will be of some importance.

SUMMARY

1. A method is described by means of which the surface area of puparia of *Drosophila melanogaster* may be measured.

2. Measurement of almost 200 puparia showed that the relationship between surface area, per mg., and body weight could best be expressed in the form

of the equation $S = 7{\cdot}7049 - 2{\cdot}1099X$, where S = surface area, sq. mm. per mg. wet weight, for prepupae of mean wet weight X mg. As the standard error of estimate, $\pm 0{\cdot}117$, is equal to only 2·2% of the mean surface area per mg., the surface area can be accurately estimated from the wet weight.

3. The prepupal oxygen consumption, per mg. wet weight, is shown to decrease steadily with increasing body weight; with an increase in mean wet weight from 0·847 to 1·700 mg., the oxygen consumption, per mg., decreases by about 50%.

4. Utilizing the above regression equation, the surface area of prepupae of known oxygen consumption was estimated and thus the oxygen consumption per sq. mm. of body surface. These values show no significant variation with increasing body weight, so that it can be concluded that the oxygen consumption of prepupae of *D. melanogaster* is proportional to the surface area.

The experiments on which the present paper is based were carried out in the Department of Zoology, University College, London, during 1935–7. It is a pleasure to thank Prof. Watson, F.R.S., for his interest in the work, and to acknowledge the great debt I owe to the encouragement I then received from Dr N. H. Howes.

REFERENCES

Abercrombie, W. F. (1936). *J. Morph.* **59**, 91.
Alpatov, W. W. (1930). *Biol. Bull. Woods Hole*, **58**, 85.
Benedict, F. G. (1938). *Publ. Carneg. Instn*, no. 503.
Benedict, F. G. & Talbot, F. B. (1921). *Publ. Carneg. Instn*, no. 302.
Bliss, C. I. (1926). *J. Gen. Physiol.* **9**, 467.
Bodenheimer, F. S. (1929). *Z. angew. Ent.* **15**, 435.
Bodine, J. H. (1921). *J. Exp. Zool.* **32**, 137.
Bodine, J. H. & Orr, P. R. (1925). *Biol. Bull. Woods Hole*, **48**, 1.
Bowden, F. P. & Rideal, E. K. (1928). *Proc. Roy. Soc.* A, **120**, 58.
Bowden, F. P. & O'Connor, E. A. (1930). *Proc. Roy. Soc.* A, **128**, 317.
Butler, C. G. & Innes, J. M. (1935). *Proc. Roy. Soc.* B, **119**, 297.
Clare, M. R. (1925). *Biol. Bull. Woods Hole*, **49**, 440.
Dobzhansky, Th. & Poulson, D. F. (1935). *Z. vergl. Physiol.* **22**, 473.
Ellenby, C. (1937). *Nature, Lond.*, **140**, 853.
Ellenby, C. (1938). *Proc. Zool. Soc. Lond.* A, **108**, 525.
Franekel, G. & Herford, G. V. B. (1938). *J. Exp. Biol.* **15**, 266.
Huxley, J. (1932). *Problems of Relative Growth.* Methuen.
Loewenthal, H. (1923). *Arch. Zellforsch.* **17**, 86.
Needham, J. (1934). *Biol. Rev.* **9**, 79.
Orr, P. R. (1925). *J. Gen. Physiol.* **7**, 730.
Pfaundler, M. (1921). *Arch. Anat. Physiol.* (*Physiol.*), **188**, 273.
Poulson, D. F. (1935). *Z. vergl. Physiol.* **22**, 466.
Robertson, C. W. (1936). *J. Morph.* **59**, 351.
Rubner, M. (1913). *Arch. Anat. Physiol.* (*Physiol.*), **180**, 240.
Simanton, W. A. (1933). *Ann. Ent. Soc. Amer.* **26**, 247.
Snedecor, G. W. (1938). *Statistical Methods.* Ames, Iowa: Collegiate Press, Inc.
Strasburger, E. H. (1935). *Drosophila melanogaster* Meig. Berlin: Springer.
Teissier, G. (1929). *C.R. Soc. Biol., Paris*, **100**, 1171.
Teissier, G. (1931). *Trav. Sta. biol. Roscoff*, **9**, 29.
Trager, W. (1935). *J. Exp. Zool.* **71**, 489.
Vernon, W. H. J. (1926). *J. Chem. Soc.* p. 2273.
Vernon, W. H. J. (1935). *Trans. Faraday Soc.* **31**, 1673.
Whitney, R. J. (1942). *J. Exp. Biol.* **19**, 168.
Wingfield, C. A. (1939). *Proc. Zool. Soc. Lond.* A, **109**, 103.
Wolsky, A. (1938). *J. Exp. Biol.* **15**, 225.

SOME OBSERVATIONS ON THE PHYSIOLOGY AND PHARMACOLOGY OF THE NERVE ENDINGS IN THE CROP AND GIZZARD OF THE EARTHWORM, WITH SPECIAL REFERENCE TO THE EFFECTS OF COOLING

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(With Ten Text-figures)

The use of the crop and gizzard of the earthworm as a convenient smooth-muscle preparation was first suggested by Mines (1907). The pharmacological properties of this preparation have recently been described by Wu (1939). His experiments and Millott's (1943) have shown that the digestive tract of the earthworm, including the crop and gizzard, is reciprocally innervated. The nerve fibres concerned are cholinergic in the augmentor and adrenergic in the inhibitory nerves.

This paper deals with certain changes in the behaviour of this muscle observed after cooling. Dikshit (1938) has shown that, after cooling strips of mammalian intestines for 3–4 days, the nerve endings present in them lose the property of synthesizing and liberating acetylcholine (ACh.). The muscle fibres, on the other hand, are more resistant to cold and are still able, at this stage, to contract in response to added acetylcholine. Feldberg & Solandt (1942) have used this method to study the reactions of smooth muscle in the rabbit's intestine when deprived of the function of its nervous elements. Corresponding experiments on the crop and gizzard before and after cooling are described in this paper. In addition, preparations made from worms caught during a long spell of cold weather as well as from worms kept for several days in the refrigerator were examined in order to ascertain whether they showed changes similar to those observed in the cooled preparations.

METHODS

The earthworms *Lumbricus terrestris* and *Allolobophora longa* were used in most of the experiments. The body wall was opened in the mid-dorsal line by a longitudinal incision. The transverse septa were cut and the crop and gizzard were freed by dissection from the dorsal blood vessel and from the nerve cord ventrally. The contents of the lumen were not usually emptied. A ligature was tied round each end of the crop and gizzard. The preparation was freed by a cut above and below the ligatures and suspended in a 2 c.c. bath, in oxygenated Ringer's solution (NaCl 6·5 g.; KCl 0·14 g.; $CaCl_2$ 0·12 g.; NaH_2PO_4 0·01 g.; $NaHCO_3$ 0·2 g.; distilled water to 1000—*p*H 7·4–7·5). The bath was of the type described by Wells (1937). It was emptied by overflow and was fitted with a glass two-way tap so as to be able to switch over rapidly from the Ringer's solution to solutions containing various drugs. In some experiments the Ringer's solution was diluted with one-third its volume of distilled water to make it isotonic with the body fluids of the earthworm (Adolph, 1927), but it was found, as reported by Wu and Millott, that frog Ringer's solution was equally satisfactory. A rapid switch over from one Ringer's solution to the other had no effect whatsoever. The contractions of the muscle were recorded by a sensitive 'balance-type' isotonic lever. In this the friction at the fulcrum was reduced to a minimum by the use of a knife-edge on an agate. It was possible, by means of a fine adjustment over the bearing, to raise or lower, as necessary, the centre of gravity of the lever and so to set it just below the fulcrum. The load on the muscle could be varied by means of another fine screw-adjustment on the beam of the lever. Minimal loads and a magnification varying between 20 and 25 × were used.

Preliminary experiments showed that the friction of the writing-point on the drum may sometimes overcome the pull of the muscle. This difficulty was avoided by the use of a very light constant-writing device of the type shown in Fig. 1. This consisted of a fine steel wire bent twice at right angles and coated at its tip with a smooth bead of sealing-wax. It was inserted into a short glass capillary tube at the end of the lever and hung loosely from it. The lever was bent slightly upwards at its end to prevent the wire from falling out of the capillary tube whenever this was below the horizontal position. The centre of gravity of the bent wire tended to keep the writing point on the drum, but owing to the fact that it was freely mobile inside the glass capillary tube it was able to override any small irregularities on the smoked paper which would otherwise have caused

it to stick. In each experiment the additional precaution was taken of comparing, with the naked eye, the excursion of the lever before and after the writing-point was applied to the drum in order to make sure that the size of the contractions was not reduced by friction.

Fig. 1. Diagram showing the type of constant-writing device referred to in the text.

The experiments were carried out on three types of preparation:

(1) Preparations made from worms previously kept at room temperature (17–23°C.) for several days ('warm' preparations).

(2) 'Cooled' preparations, which were also made from worms kept at room temperature. The preparations were then left in the refrigerator for a varying number of hours before the experiment, in Ringer's solution of the same osmotic pressure as the body fluids of the earthworm and containing 0·2–0·5 g. of glucose per litre.

(3) 'Cold-worm' preparations, which were obtained from worms caught in winter or kept in captivity at temperatures then prevailing out of doors. In a few experiments the whole worm was kept in the refrigerator for several days before the experiment.

RESULTS

(1) *The spontaneous activity of the crop and gizzard*

(*a*) *'Warm' preparations.* The crop and gizzard contract rhythmically, as shown by Mines and by Wu. This activity persists for many hours and is not affected by the absence of glucose or by oxygenation. The following observations suggest that these movements are peristaltic in nature. By 'peristalsis' we mean a propagated wave of contraction depending on a local nervous reflex. It was not possible to show, in this case, whether the waves of contraction were preceded by waves of relaxation, as in Bayliss & Starling's experiments (1899).

The waves of contraction can be followed under the binocular dissecting microscope, and even with the naked eye they can be seen to start in the crop and to move down into the gizzard. There is usually a short time-lag between the contraction of the crop and that of the gizzard. This is shown in Fig. 2, where the smaller contractions of the crop were marked *C* and those of the gizzard *G*. The difference in the height of the two contractions corresponds to the difference in size of the two muscles. The tracings in Fig. 2 were taken from different experiments. Fig. 2 A shows that the time-lag between the two contractions may sometimes vary from cycle to cycle in the same preparation. In Fig. 2 B there is a greater separation of the two components. This is due partly to the fact that the tracing was taken with a faster drum and partly to a longer time-interval between the two contractions which, in this preparation, was fairly constant. There is also, sometimes, a notch on the relaxing phase of the cycle (asterisks in Fig. 2 A, C), suggesting that relaxation may not start in the gizzard before it is complete in the crop. The process recurs rhythmically two or three times a minute. The presence of earth in the lumen would seem to provide the continual stimulus for this activity, for the reason that on the few occasions when the lumen was left open at the lower end of the gizzard the contractions became irregular and progressively weaker as the contents of the preparation were expelled. On introducing a small glass bead into the gizzard, the contractions became more vigorous and regular again and led to the eventual expulsion of the bead, with a subsequent diminution of spontaneous activity.

In the ordinary preparations, in which the lumen was closed at both ends, it was noticed that, whereas at the beginning of the experiments the earth was uniformly distributed throughout the crop and gizzard, towards the end the crop had usually emptied itself into the gizzard. At this stage the former no longer participated in the rhythmic activity of the preparation, which consisted of single smooth contractions in the gizzard. These are shown in Fig. 2 D.

Further evidence suggesting that the nature of these movements is peristaltic is provided by observations made on the cooled crop and gizzard as well as by experiments with nicotine.

On several occasions a few millimetres of intestine were included in the preparation. At first the intestine was practically empty, but after a time it became increasingly distended with earth driven down into it by vigorous contractions in the gizzard. It was possible to see the earth moving down into the intestine with each contraction. The eventual accumulation of earth produced a great increase in pressure in the lumen, which led to a gradual thinning of the wall of the intestine and an increase in its transparency. Eventually, when stretched to the limit, the intestine burst during a contraction of the gizzard. Before this happened, it was noticed that contraction in the gizzard was attended by a further rise in pressure inside the intestine, as shown by the appearance of sacculations in its wall. Despite the existence of this positive pressure inside the intestine it was observed that there was never any escape of earth back into the gizzard in the intervals between

the contractions of the latter. This is suggestive of the presence of a functional sphincter between the gizzard and the intestine. There is no evidence in the literature for the existence of such a sphincter, but on histological examination of that region it was found to consist almost entirely of circular muscle. A few nerve cells were seen in the substance of the muscle (see p. 54).

In several experiments records were made of the movements of the circular muscle, which is particularly well developed in the proximal three-quarters of the gizzard. This muscular ring was slit open and suspended in the bath. The slight stretch on the muscle appeared to provide the same stimulus as a bolus. The rhythmic contractions, which went on for many hours, are shown in Fig. 2 E. The frequency of these contractions was the same as that of the rhythmic activity recorded in the whole crop and gizzard.

suspension in Ringer's solution at room temperature. If the cooling was prolonged, the spontaneous activity of the crop and gizzard disappeared by the third or fourth day. The muscle then showed only slight variations in tone which were slow and irregular (Fig. 5).

At lower temperatures (−2–0°C.) the disappearance of rhythmic activity was seen earlier. Cooling affected the spontaneous movements of the circular muscle in the same way (Fig. 6 B).

The absence of autonomous movements after cooling might indicate an impairment of the muscle fibres, but experiments with ACh. (see p. 49) show that the excitability of the muscle is not affected to any great extent by such treatment.

(*c*) '*Cold-worm*' *preparations*. The crop and gizzard of 'cold' worms behave in many respects like the cooled preparations. There is an impairment of rhythmic activity which varies with the period of

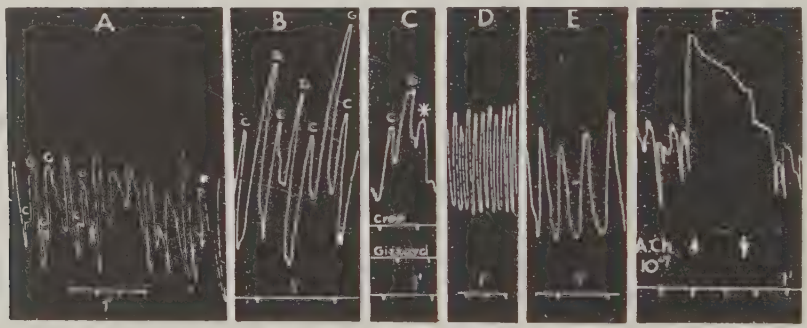

Fig. 2. Records of the rhythmic contractions of the crop and gizzard of the earthworm. A, B, C, D, E, F, all from different preparations. For explanation see text. In C the contractions were timed separately in the crop and in the gizzard. Their duration is shown in the two middle lines. Time in minutes throughout.

In the mammalian intestine peristalsis is, according to Bayliss & Starling (1899), a function of both muscular layers. It would seem that this is also the case in the earthworm. It has already been stated that the frequency of the spontaneous movements in the circular and longitudinal muscle layers is the same. The contractions in the two layers could occur either simultaneously or out of phase with each other. Simultaneous records of the rhythmic activity in the two layers were never obtained, but from naked-eye observation both layers appeared to contract simultaneously. This was also the case in the experiments in which the lumen was left open at the lower end of the gizzard. In such preparations the expulsion of earth or of a glass bead, which involved the contraction of the circular muscle, occurred at the height of a contraction in the longitudinal muscle.

(*b*) '*Cooled*' *preparations*. After short periods of moderate cooling (5–7°C. for 1 or 2 days) there was no apparent change in rhythmicity on subsequent

exposure and the severity of the cold. Finally, the spontaneous activity disappears. The preparation in the experiment shown in Fig. 6 A was made from a worm which had been kept for 7 days in the refrigerator at 2–3°C. When taken out of the cold the worm was still capable of vigorous crawling movements on the bench. The nerves to the musculature of the body wall would therefore seem to be little affected by cold. Nevertheless, the crop and gizzard, when suspended in oxygenated Ringer's solution, were found to have lost their spontaneous activity. This did not return after the subsequent addition of glucose. Circular muscle preparations behaved similarly. The excitability of the muscle was in each case unimpaired, as shown by the effect of ACh. (see p. 49).

In less extreme cases, when the crop and gizzard were suspended, there was a short initial period of rhythmic activity attended by a gradual relaxation of the muscle. During this period the contractions

of the preparation became progressively weaker and less frequent until, finally, they disappeared. The muscle was then fully relaxed. The disappearance of spontaneous activity seemed to be hastened by the absence of glucose and oxygen. Subsequent administration of glucose or of oxygen or both was usually ineffective, but on one occasion oxygen brought about a return of weak spontaneous contractions which disappeared again when oxygenation was stopped.

In view of these findings the worms from which 'warm' preparations were made were kept at room temperature for several days before use during the winter.

(2) *The action of drugs*

(*a*) *Acetylcholine.* The observations made by Wu that warm preparations are extremely sensitive to ACh. and that *Lumbricus* is more sensitive than *Allolobophora*, were confirmed. For *Allolobophora* the threshold varied between 10^{-7} and 10^{-9}, and for *Lumbricus* between 10^{-8} and 10^{-10}. In the presence of eserine (1 in 500,000) responses were obtained from *Lumbricus* preparations to even smaller concentrations of ACh. (10^{-11}). The contraction produced by ACh. starts immediately and lasts until the ACh. is washed out (Fig. 2 F). This effect is inhibited by atropine, although the latter, when given alone, does not abolish the rhythmic activity of the crop and gizzard. The effect of ACh. on the circular muscle is the same as on the whole preparation.

It has been stated that a glass bead introduced into a gizzard, the lumen of which was left open at its lower end, produced vigorous contractions. If a large bead is used in such an experiment, these contractions are insufficient to bring about its expulsion, but on addition of ACh. to the bath the forcible contraction produced is strong enough to expel the bead.

The response to ACh. is still present in cooled preparations long after the disappearance of all rhythmic activity. The preparations appeared to be slightly less sensitive than warm preparations, but reacted regularly and repeatedly to ACh., with contractions of constant height (Fig. 5 A). In several instances the ACh. contraction started after a short latent period of a few seconds. Similar results were obtained with cooled preparations of the circular muscle (Fig. 6 B, 2). Cold-worm preparations which showed no spontaneous activity (see p. 48) also retained their excitability to ACh. (Fig. 6 A, 4).

(*b*) *Eserine.* Eserine enhances the action of ACh. when the two are given together. According to Wu, eserine alone causes an increase in frequency of the rhythmic contractions and a slowly developing rise in tone. In our experiments the change in frequency was either absent or present only in the first few minutes after the administration of eserine. The main effect was a very slow increase in tone. This response is quite different from the immediate contraction seen when ACh. is added to the bath, and is such as would be produced by a gradual accumulation of ACh. resulting from the inhibition of choline-esterase.

(*c*) *Adrenaline.* Wu described a twofold action of adrenaline on the crop and gizzard. The effect on the muscle varies with the dose of adrenaline, being augmentor with small (10^{-7}) and inhibitory with larger doses (10^{-5}). This result was confirmed on the warm preparations. The large inhibitory doses reduce the size of a contraction produced by ACh.

In cooled preparations the response to adrenaline was altered. The contraction produced by ACh. was still reduced by large doses of adrenaline, but, if given alone, adrenaline had no action whatsoever whether in small or large doses (Fig. 5 C, D). In cold-worm preparations adrenaline was also ineffective. It has been stated that some cold-worm preparations exhibited an initial period of rhythmic activity followed by 'silence'. On such preparations the motor and inhibitory effects of adrenaline could be demonstrated during the initial period of spontaneous activity. With the disappearance of the latter, both actions of adrenaline were lost.

(*d*) *Potassium.* Wu's experiments have shown that potassium stimulates the crop and gizzard; with large doses this effect is followed by inhibition. These results were confirmed on warm preparations. Fig. 3 A shows the contraction produced by a sudden fifteenfold increase in the potassium content of the Ringer's solution to a final concentration of 0·21 % KCl. In the same experiment the augmentor action of potassium was enhanced by eserine (Fig. 3 B), but was not affected by atropine.

Cooling produced several alterations in the action of potassium. The first change that occurred, in cooled preparations which still retained their spontaneous activity, was a disappearance of the stimulating effect of KCl. The inhibitory action of potassium was still present (Fig. 3 C) and was produced by doses of KCl which before cooling had produced contraction on the same preparation. This occurred in the experiment shown in Fig. 3 C after cooling for 17 hr. at 2–3° C., and in another experiment after 66 hr. at 7° C. The preparation of Fig. 3 C was returned to the refrigerator and re-examined 55 hr. later. There was then no sign of any rhythmic activity, and it was found that the inhibitory action of KCl had also disappeared. Fig. 5 B, which is from the same preparation, shows that potassium had then no effect whatsoever on the muscle, although this still responded normally to ACh. (Fig. 5 A). Moreover, potassium did not affect in any way the contraction produced by ACh. In one instance potassium had no effect by itself on a cooled preparation, but in the presence of eserine it gave rise to a small rapid contraction which was not sustained.

The effect of KCl on the cold-worm preparations was similar to that on the cooled preparation. When spontaneous activity was absent, the muscle no

longer reacted to potassium (Fig. 6 A, 1 and 3). In those cold-worm preparations in which, during the course of the experiment, rhythmicity was lost together with the excitability to adrenaline, the response to KCl was similarly affected. At such a stage both the stimulating and the inhibitory actions of potassium were found to have disappeared, an immediate and vigorous contraction. The contracted muscle exhibited strong rhythmic activity and a gradual return, within 3 min., to its original tone.

With higher concentrations of $CaCl_2$ (0·12%), the depression was more pronounced. The muscle relaxed and its spontaneous activity was usually ar-

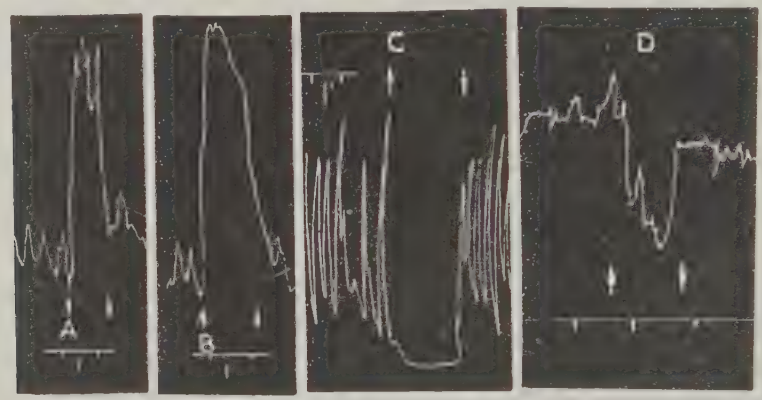

Fig. 3. A and B from the same warm preparation. Between the arrows: A, 0·21% KCl; B, 0·21% KCl and 1 in 200,000 eserine sulphate together; C, inhibitory effect of 0·14% KCl on a different preparation, cooled for 17 hr. at 2–3°C.; D shows the effect of 0·21% KCl on another preparation, from a cold worm.

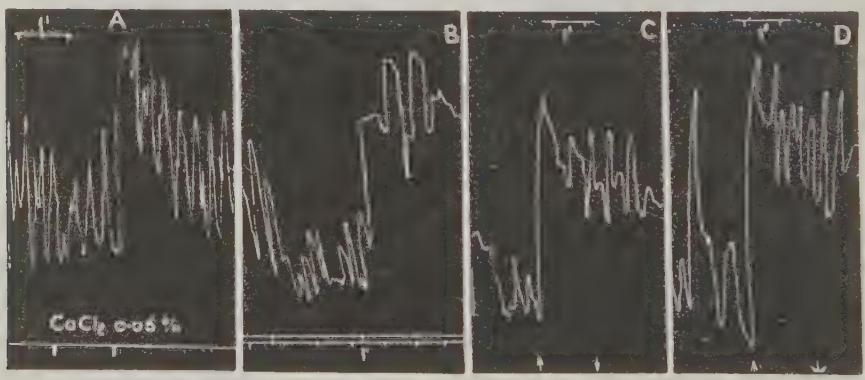

Fig. 4. Warm preparations. Between the signals: A, 0·06% $CaCl_2$; B, 0·14% KCl and 0·12% $CaCl_2$ together. C and D from another preparation; C, ACh. chloride 10^{-7}; D, ACh. chloride 10^{-7} and 0·12% $CaCl_2$ together.

but, on one occasion, only the stimulating action of KCl was absent after the disappearance of spontaneous activity and the effect of potassium was then inhibitory (Fig. 3 D).

(*e*) *Calcium.* The effect of calcium is inhibitory. Fig. 4 A shows the slight diminution in rhythm produced by 0·06% $CaCl_2$. This represents a fivefold increase in the calcium content of the Ringer's solution. On washing out the excess calcium there ensued rested. After washing the calcium out, there was a return of rhythmic activity, usually with a small increase in tone, but the latter was sometimes absent. When potassium and calcium were given together the inhibitory effect of the latter prevailed. Fig. 4 B shows the effect of raising simultaneously the potassium content of the Ringer's solution 15 times (0·21% KCl) and the calcium 10 times (0·12% $CaCl_2$). The inhibition was almost as deep as that

produced by an excess of calcium alone, and was followed, on washing out, by a prompt and vigorous contraction of the muscle. When calcium and ACh. were administered together, the stimulating effect of the latter prevailed and was usually little affected (Fig. 4 C, D). On one occasion, the preparation being rather insensitive to ACh., the immediate contraction produced by a threshold dose of ACh., when given alone, was delayed by the simultaneous addition of calcium. On 'cooled' and 'cold-worm' preparations, which showed no rhythmic activity, calcium was ineffective (Figs. 5 E and 6 A, 2) and did not influence the response to ACh. when the two were given together (Fig. 5 F). In one cold-worm preparation which exhibited rhythmic activity for a short time after suspension, the addition of calcium produced inhibition which was followed, on washing out, by the disappearance of spontaneous activity. The failure of rhythmic activity appeared in this case to be hastened by the administration of calcium. Subsequently, calcium had no effect.

(*f*) *Barium.* On warm preparations barium produced a well-marked contraction (Fig. 7 A) which was completely inhibited by calcium (Fig. 7 C). The barium response was diminished by cooling, and eventually disappeared. This is illustrated in Fig. 7 A, B, which were obtained from the same preparation before and after cooling. The effect of $BaCl_2$ 10^{-4} on the warm crop and gizzard is shown in Fig. 7 A. The preparation was then cooled at 2–3°C. and remained in the cold for 72 hr. Rhythmic movements were absent. Between the arrows: A, ACh. chloride 2×10^{-8}; B, 0·28% KCl; C, adrenaline HCl 10^{-7}; D, adrenaline HCl 10^{-5}; E, 0·12% $CaCl_2$; F, ACh. chloride 2×10^{-8} and 0·12% $CaCl_2$ together.

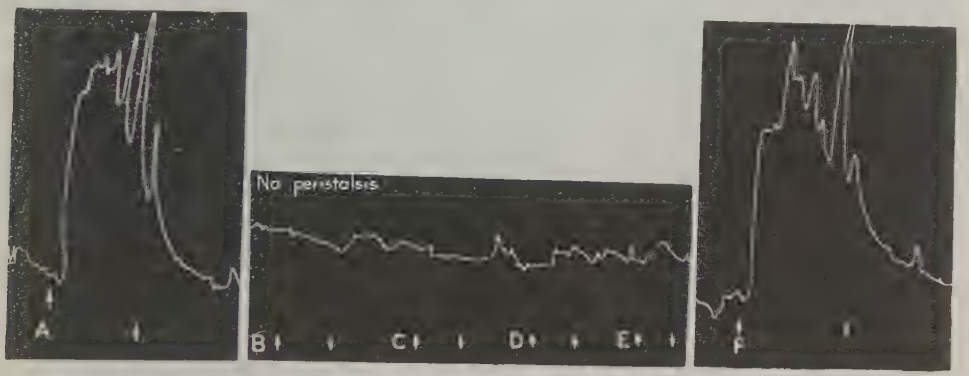

Fig. 5. Records from a cooled preparation. In this experiment the crop and gizzard were cooled for 72 hr. at 2–3°C. Rhythmic movements were absent. Between the arrows: A, ACh. chloride 2×10^{-8}; B, 0·28% KCl; C, adrenaline HCl 10^{-7}; D, adrenaline HCl 10^{-5}; E, 0·12% $CaCl_2$; F, ACh. chloride 2×10^{-8} and 0·12% $CaCl_2$ together.

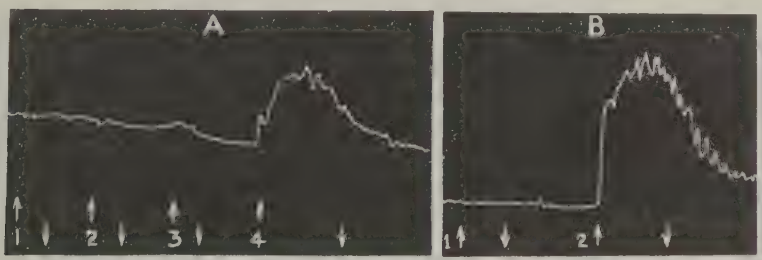

Fig. 6. A, preparation from a worm which was kept for 7 days at 2–3°C. Note absence of rhythmic activity. Between the arrows: 1, 0·14% KCl; 2, 0·12% $CaCl_2$; 3, 0·28% KCl; 4, ACh. chloride 10^{-7}. B, circular muscle preparation from a cooled crop and gizzard showing absence of rhythmic activity. Between the arrows: 1, 0·14% KCl; 2, ACh. chloride 10^{-5}.

examined after 72 hr. when all rhythmic activity had disappeared. At this stage the excitability to ACh. was still present (Fig. 5 A), but $BaCl_2$ 10^{-4} had no effect. The dose of barium was increased to 2×10^{-4} and the small response then obtained is shown in Fig. 7 B.

(*g*) *Cocaine.* Millott has described an experiment in which he painted the oesophagus of an earthworm with 5% novocaine and observed immediate relaxation and cessation of rhythmic activity. He left open the question whether this result was due to an action of the drug 'only on nervous tissue and not

also on the muscles' (Millott, 1943). The present experiments show that cocaine has, in addition to the action described by Millott, a stimulating effect on the crop and gizzard and that its action depends upon its concentration in the bath. In warm preparations cocaine, in concentrations up to 1 in 200, its action on warm preparations in that it caused cessation of peristaltic activity without loss of excitability to ACh. Fig. 8 shows the diminution in rhythmic activity produced by nicotine (10^{-4}). In this experiment there was a gradual decrease in the size and frequency of the rhythmic contractions

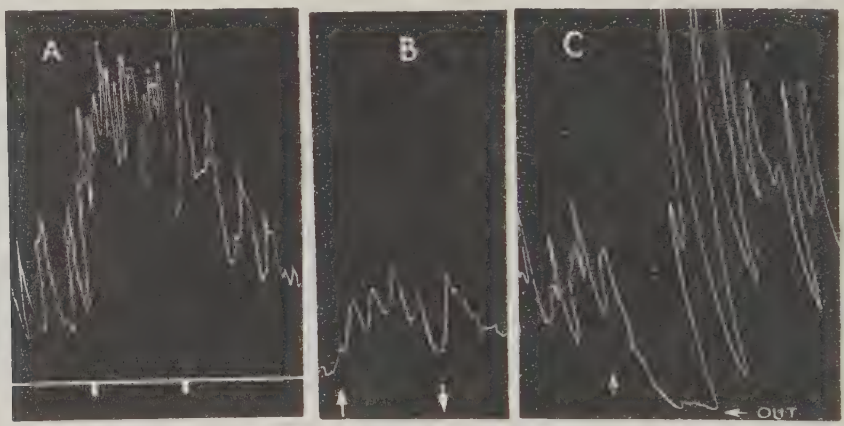

Fig. 7. A and B from a 'warm' preparation (from the same experiment as Fig. 5). A, before cooling, $BaCl_2$ 10^{-4}; B, after 72 hr. cooling at 2–3° C., $BaCl_2$ 2×10^{-4}. C from another preparation: $BaCl_2$ 10^{-4} and 0·12 % $CaCl_2$ together.

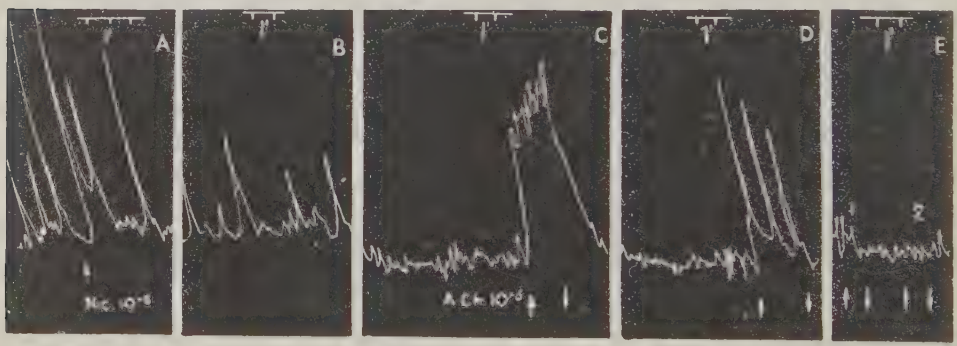

Fig. 8. The effect of nicotine on a warm preparation. A, nicotine hydrogen tartrate 10^{-5} at the arrow. Between A and B nicotine increased to 10^{-4} and left in the bath to the end of the experiment. B, 20 min. later. C, 30 min. later. ACh. chloride 10^{-6} between the arrows. D, 40 min. later. 0·14 % KCl between the arrows. E, 45 min. later: 1, $BaCl_2$ 10^{-4}; 2, $BaCl_2$ 2×10^{-4}.

produced an increase in size of the rhythmic contractions. The inhibitory effect of cocaine became manifest only with higher concentrations. An arrest of rhythmic activity was produced by increasing the concentration of cocaine to 1 in 100. But in this condition the preparation was insensitive to ACh., indicating an impairment of the muscle fibres.

(*h*) *Nicotine.* Nicotine differed from cocaine in after the introduction of nicotine into the bath. At the end of half an hour the depression produced by nicotine was complete. At this stage there were no peristaltic contractions, but the muscle still responded normally to ACh. (Fig. 8 C). The response to potassium was still present, but slightly reduced (Fig. 8 D). Nicotine abolished the effect of barium 10^{-4} (Fig. 8 E, 1) and 2×10^{-4} (Fig. 8 E, 2).

(3) *The release of acetylcholine during rhythmic activity*

In order to demonstrate the release of ACh. from the crop and gizzard, a warm preparation was placed for 20 min. in 5 c.c. of oxygenated Ringer's solution containing 1 in 10,000 of eserine. During this period eserine in the sample. In another experiment this possibility was obviated by assaying the sample on a previously eserinized preparation. The results were similar. The other half of the bath fluid was treated with *N*/10 NaOH for 20 min. at room temperature, then neutralized and tested, at *D*, which shows that the sample had become inactive. The active principle

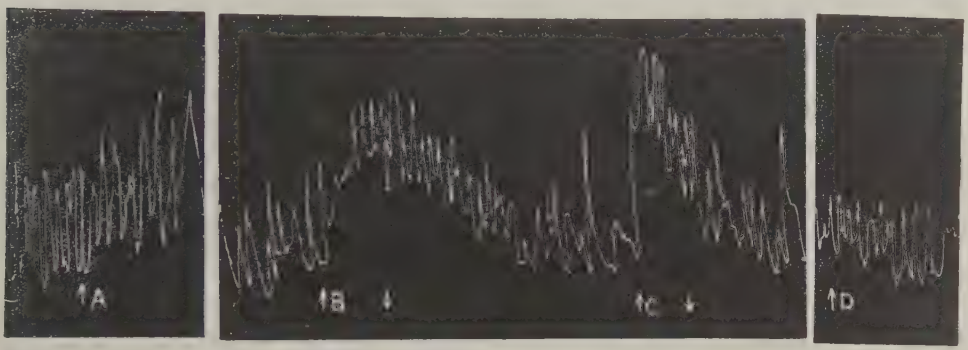

Fig. 9. A warm preparation. Assay of the eserinized bath fluid from another crop and gizzard. For explanation see text. The bath fluid was changed between each sample. A, ACh. chloride 10^{-8}; B, active sample from another crop and gizzard; C, ACh. chloride 5×10^{-8}; D, sample B after alkali inactivation.

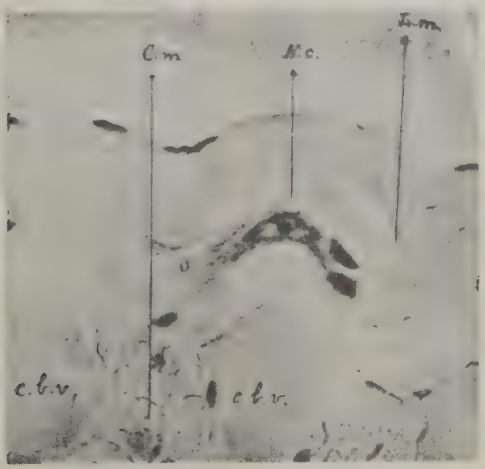

Fig. 10. Longitudinal section showing a nerve cell in the enteric plexus of the crop and gizzard. ×720. Haemalum and eosin. *C.m.* circular muscle; *c.b.v.* commissural blood vessels; *L.m.* longitudinal muscle; *N.c.* nerve cell.

the muscle showed active contractions. At the end of 20 min. the bath fluid was removed and half of it was tested at once on another warm preparation. It caused an immediate contraction of the type produced by ACh. (Fig. 9 B) and different from the slowly developing effect produced by eserine. It could not therefore have been due to the presence of in the bath fluid is therefore, like ACh., sensitive to alkali. The activity of the bath fluid when assayed against ACh. was found to lie between 10^{-8} (Fig. 9 A) and 5×10^{-8} (Fig. 9 C). On repeating the experiment with a cold-worm preparation which did not exhibit spontaneous activity, a release of ACh. could not be demonstrated.

(4) *Histological observations*

Sections were cut through the crop and gizzard and examined for the presence within the muscle of nerve cells. These were found, in small clusters, in a position corresponding to that of Auerbach's plexus, between the longitudinal and circular muscle layers and just peripheral to the commissural blood vessels within the circular muscle (Fig. 10). They are seen most clearly in the gizzard, where the two muscle layers are thickest and best differentiated. The circular muscle layer was found to be free from nerve cells throughout its thickness, except at the lower end of the gizzard (see p. 48). A number of small, darkly stained nuclei are present in the substance of the muscle at the outer edge of the muscle fibres, but they could not be mistaken for nerve cells. The cells in the enteric plexus are characteristically multipolar, some with long visible processes, and it is possible to distinguish clearly Nissl's granules and the nucleolus in some of them (Fig. 10, *N.c.*). They are rather similar in texture and staining properties to the nerve cells present in the ganglia of the ventral nerve cord, but slightly larger in size.*

DISCUSSION

The experiments described in the preceding section show that the rhythmic activity of the preparation is dependent upon the integrity of some nervous mechanism within the crop and gizzard. This possibility was suggested by the propagation of the contraction waves from the crop to the gizzard and their dependence on the presence of earth or of a glass bead inside the lumen. Indeed, it would be difficult to conceive such co-ordination between the movements of crop and gizzard without some nervous intervention. This suggestion was strengthened by the effect of cooling and by the experiments with nicotine. In both cases there was a disappearance of the spontaneous movements of the crop and gizzard without a loss of excitability in the muscle as shown by its reactions to ACh. In view of the fact that cocaine, unlike nicotine, produces an impairment of the muscle, the disappearance of rhythmic activity in the cocainized preparation cannot be used as additional evidence on this point.

The spontaneous movements of the crop and gizzard are therefore more akin to the peristaltic waves in the mammalian intestine than to the rhythmic 'pendulum movements' displayed by the longitudinal muscle of the latter. This peristaltic activity involves a reflex mechanism for which the appropriate stimulus is a distension of the lumen. In circular muscle preparations this is imitated by the stretch produced by the lever. The absence of rhythmicity after cooling would appear to be associated with loss of nervous function and the inability to synthesize ACh. Our findings with nicotine are similar to those of Bayliss & Starling (1899) on the mammalian intestine. The effect of nicotine would be due to an interruption of the reflex arc by a gradual paralysis of the parasympathetic nerve cells. Histological evidence for the existence of such cells in the crop and gizzard has been scanty. Various authors quoted in Stephenson's monograph (1930) have described a fine network of nerve fibres in the gut of the earthworm. Hess (1925) alone mentions 'gangliated thickenings in the enteric plexus which are probably associated with peristalsis'. In our own histological sections the presence of nerve cells could be clearly demonstrated. It is significant that these lay in the anatomical plane which corresponds to the situation of Auerbach's plexus in the mammalian intestine.

It was assumed that the peristaltic reflex leads to a release, by the nerve endings, of ACh., which is the ultimate stimulus for the contraction of the muscle. The enhancement of this activity by eserine, with its characteristic slow development, increased the likelihood of such a release. Evidence for the liberation of ACh. is clearly provided in the experiments in which the bath fluid from an eserinized crop and gizzard was assayed on another preparation. The absence of any such release in cold preparations is in accordance with the observations of Dikshit on the mammalian intestine.

The fact that in the earthworm peristalsis is not inhibited by doses of atropine, which render the muscle insensitive to added ACh., would indicate that ACh. is released by the nerve endings in such close proximity to the muscle fibres that atropine is unable to interfere with its action. This kind of reasoning has been applied to other discrepancies between the effects of nerve stimulation and of ACh. in the presence of atropine (Dale & Gaddum, 1930). In this respect too the crop and gizzard of the earthworm behave like the mammalian intestine, in that peristaltic reflexes are not abolished by atropine (Bayliss & Starling, 1899).

Although cooling did not affect the excitability of the muscle to ACh., the response to other drugs was considerably altered by such treatment. This suggests that a nervous mechanism is involved in the action of these drugs. Of the four substances the action of which was affected by cooling, barium had, on warm preparations, a stimulant action only, whereas potassium, adrenaline and calcium had both motor and inhibitory effects. Prolonged cooling abolished not only the stimulating but also the inhibitory effects of these substances. If we assume that the disappearance of both these effects is associated with the loss of nervous function, we are forced to make the further assumption that more than one nervous mechanism is involved in the action of these drugs. There is good evidence for the existence of a double nerve supply to the earthworm gut con-

* We are indebted to Mr E. N. Willmer for examining our sections and confirming the nervous nature of these cells.

sisting of antagonistic cholinergic and adrenergic nerve fibres. The results of our experiments with these drugs can be interpreted by assuming that they act in warm preparations on one or the other set of nerve fibres or their endings.

Adrenaline. The twofold action of adrenaline on warm preparations may be explained by the fact that the sympathomimetic or inhibitory effect of adrenaline does not appear below a certain threshold, which is of the order of 10^{-6}. The stimulating action of adrenaline which is seen below that threshold would seem to be due to an improvement in acetylcholine-transmission at the synapse in the peristaltic reflex arc and at the neuromuscular junction. A similar effect of adrenaline on skeletal muscle has been described by Bülbring & Burn (1939). The disappearance of this action of adrenaline in cooled preparations would be due to the fact that in these acetylcholine-transmission is absent. The inhibitory action of adrenaline is not apparent after cooling, for the sole reason that the muscle is toneless in the absence of ACh. But if a large dose of adrenaline is administered in the middle of a contraction produced by ACh., it still brings about immediate relaxation of the cooled preparation, showing that its inhibitory action is direct on the muscle.

Potassium. The stimulating action of potassium may be accounted for by a release of ACh. from the cholinergic nerve fibres and their endings. Such an action of KCl has been shown on a wide variety of cholinergic nerve fibres and their endings (Beznàk, 1934; Brown & Feldberg, 1936; Feldberg & Guimarais, 1936; Chute, Feldberg & Smyth, 1940), and from brain slices in vitro (Quastel, Tennenbaum & Wheatley, 1936). This action of KCl disappears after prolonged cooling because the synthesis of ACh. is then abolished.

From experiments on the rabbit's intestine, Vogt (1943) concluded that potassium acts directly on the muscle fibre, since its action was not inhibited by nicotine. These observations were confirmed on the crop and gizzard, but, in view of the disappearance of the action of potassium after cooling, it is possible to give a different interpretation of this phenomenon. Nicotine does not affect the function of nerve endings, but only that of nerve cells. Thus Feldberg & Vartiainen (1934) found that although nicotine paralysed the postganglionic cells in the superior cervical ganglion, it did not interfere with the release of ACh. from the nerve endings of the preganglionic fibres when these were stimulated electrically. It is unlikely that nicotine would abolish the release of ACh. from nerve endings when this is produced by potassium. The results obtained with nicotine are therefore not at variance with the assumption that the action of potassium is mainly on the nerve endings in the crop and gizzard, although it is possible that under normal conditions the nerve cells may participate to a certain extent in this response as there was a slight diminution in the effect of potassium after nicotine.

The view that potassium stimulates the muscle by virtue of a release of ACh. is supported by the fact that the response to KCl is potentiated by eserine. That it is not inhibited by atropine would receive the explanation already given for the persistence of peristalsis after atropine. These findings are similar to those of Vogt (1943), who showed that the contraction produced by sodium lactate and hypertonic NaCl in the circular muscle of the rabbit's intestine is due to a stimulation of the cells of Auerbach's plexus. Yet the actions of these substances, although mediated by a nervous effect, and that of potassium, were not abolished by atropine.

If one assumes an analogy between the action of potassium and that of parasympathetic nerve stimulation, then one should expect them both to be similarly affected by atropine. In the mammal this is the case. Neither the effect of vagal stimulation (Bayliss & Starling, 1899) nor that of potassium (Vogt, 1943) is abolished by atropine in small doses. Whether this is also the case in the earthworm has not been investigated. Millott has reported an experiment in which the contraction of the oesophagus of an earthworm produced by electrical stimulation of its cholinergic nerve supply was abolished by an arterial injection of 50 μg. of atropine. This, in a worm of average weight 4–5 g., represents a large dose of atropine. According to Feldberg & Vartiainen (1934), such concentrations of atropine have a paralysing action on ganglion cells similar to that of nicotine. It is therefore probable that the effect observed by Millott was due to a 'nicotine block' interposed between the pre- and postganglionic fibres in the cholinergic nerves to the gut.

If cooling only affects the nervous mechanism in the crop and gizzard, then the inhibition produced by larger doses of KCl on the warm preparation is best explained by the intervention of another nervous effect, since it too is abolished by prolonged cooling. Inhibition in this case would be due to a stimulation of the sympathetic nerve fibres and endings in the preparation with a release of adrenaline. There is at present no other instance on record of such an effect on adrenergic fibres elsewhere, but that potassium can produce a liberation of adrenaline has been shown by Bacq & Rosenblueth (1934), who observed its occurrence in the suprarenal medulla.

In the warm preparations the depression produced by large doses of KCl was always seen after an initial period of stimulation. This could be accounted for by a quicker destruction of ACh. liberated from the cholinergic fibres than of adrenaline released by the adrenergic fibres. It is tempting to correlate such an explanation with the observations that although choline-esterase is present in the gut of the earthworm (Millott, 1943), no amine oxidase or any other enzyme system responsible for the destruction of the adrenaline has been found in any tissue in the earthworm (Blaschko, Richter & Schlossman, 1937). In preparations which have been cooled for a short time

it would appear that failure is more pronounced in the cholinergic than in the adrenergic fibres, with the result that the inhibition produced by potassium prevails and is now seen with lower concentrations of KCl.

Calcium. Since the effect of calcium disappears on cooling, it would seem that the inhibition it produces in warm preparations is also due to some action on the nerves. Calcium does not interfere in any way with the effect of ACh. on the muscle in cooled preparations. It therefore appears to have no action directly on the muscle fibres. A possible explanation of the inhibition produced in warm preparations by calcium is that it prevents the release of ACh. from the cholinergic nerve endings. This is in agreement with the observations of Brown & Feldberg (1936) on the preganglionic nerve endings in the superior cervical ganglion. Brown & Feldberg also found that an excess of calcium will prevent the release of ACh. which is normally produced by potassium. This would explain why calcium inhibits the effect of potassium on the crop and gizzard. The reduction in the effect of ACh. by calcium in an insensitive warm preparation may be due to the fact that, apart from its direct action on the muscle, ACh. is also capable of stimulating the cells in the enteric plexus. This action of ACh. would be abolished by calcium and also by cooling. With doses of ACh. already producing a maximal effect directly on the muscle, there is no appreciable diminution in the contraction when the contribution of the nerves to this effect is inhibited by calcium. With threshold doses of ACh., when the direct effect on the muscle is not maximal, however, the contribution of the nerves to the response would be quite appreciable, and its inhibition by calcium would account for the decrease in the size of the contraction.

It has been stated that an augmentor effect was sometimes seen immediately after the removal of calcium (Fig. 4 A). This was usually observed after low concentrations of calcium. It is possible that when the brake to the release of ACh. is removed, there is an increased liberation of ACh. which gradually subsides and which would be responsible for this effect. A similar increase in contraction after calcium inhibition was observed by Feng & Shen (1937) in the frog sciatic-gastrocnemius preparation, when the excess of calcium was removed.

The responses to calcium and to potassium and the effect of calcium removal are similar to the results obtained by Hogben (1925) on the crustacean heart and the molluscan heart and gut. This suggests the possibility that the effects he observed were also nervous in origin.

Barium. Contrary to the accepted view, the contraction produced by barium on warm preparations would appear to be due to a stimulation of the nervous mechanism in the crop and gizzard and not to a direct action on the muscle fibres, since this is absent in cooled preparations. This explanation receives further support from the experiments in which the effect of barium on warm preparations was abolished by calcium and by nicotine.

The fact that nicotine abolishes the contraction produced by $BaCl_2$ would suggest that the small doses of barium used in these experiments stimulate the parasympathetic ganglion cells of the crop and gizzard and not the nerve fibres or their endings, although bigger doses may stimulate these too.

Inhibitory responses to barium were never observed on warm preparations. In those cooled preparations in which the failure of nervous function was more pronounced in the cholinergic than in the adrenergic fibres, barium still produced a contraction of the muscle at a time when the action of potassium was predominantly inhibitory. This would be a further indication that small doses of barium do not stimulate the nerve fibres in this preparation, whether cholinergic or adrenergic, but only the nerve cells. The ganglion cells of the sympathetic nerves in the earthworm are situated in the pharyngeal plexus (Lankester, 1865) and in the ventral nerve cord (Gaskell, 1914). The only ganglion cells present peripherally in the enteric plexus belong to the parasympathetic system. Stimulation of these by very small doses of barium would therefore produce a pure augmentor response.

There is already a certain amount of evidence in the literature that larger doses of barium can stimulate cholinergic nerves and their endings. This was shown by Feng (1937), who observed, with much higher concentrations of barium, repetitive discharges in frog sciatic nerves after single-shock stimulation. A high-frequency tetanus produced, in a muscle treated with barium, a contracture of the type seen with eserine. There was the same antagonism in Feng's experiment between barium and calcium as that observed on the crop and gizzard. As he found that choline-esterase was not inhibited by barium, Feng concluded that the eserine-like action of the latter was due to an extensive leakage of ACh. from the motor nerve endings rather than to a persistence of protected ACh. Feng & Dun (1940) have also recorded, in the motor nerve of a muscle treated with barium, retrograde discharges arising from the neuro-muscular junction.

In view of these results it would seem necessary to reconsider the nature of the pharmacological action of various salts, particularly those of potassium and barium, on the mammalian intestine. In a few preliminary experiments it was possible, by cooling, to produce a disappearance of the responses to potassium and barium at a time when the excitability to ACh. was still present.

A great deal of information is now available about the properties of cholinergic nerves. The experiments described in this paper show that the behaviour of the parasympathetic nerves and their endings in the crop and gizzard well accords with the known behaviour of cholinergic nerves elsewhere.

Although the crop and gizzard of the earthworm are extremely sensitive to ACh., their use as a test object for ACh. has the great disadvantage of lacking specificity. Potassium and small doses of adrenaline produce an effect indistinguishable from that of ACh., and these substances are likely to be present in various extracts and perfusates together with ACh. These disadvantages disappear after cooling, and although there is a slight diminution of sensitivity, the preparation is so sensitive to begin with that this is of no consequence. If *Lumbricus* is used and the cooling carefully graded, the sensitivity of this preparation is of the same order as that of the dorsal muscle of the leech and the rectus abdominis muscle of the frog.

SUMMARY

1. The effect of cooling on the properties of the crop and gizzard of the earthworm has been investigated. Evidence is advanced that the rhythmic movements of the 'warm' preparation are neurogenic in origin and peristaltic in nature. They are abolished by nicotine and by cooling, but not by atropine.

2. Acetylcholine contracts the muscle in the crop and gizzard. This effect is abolished by atropine. The excitability of the muscle to acetylcholine is not lost after cooling.

3. Peristalsis is accompanied in the 'warm' preparation by a continual liberation of acetylcholine. This is absent in cold preparations. The disappearance of rhythmic activity in these is associated with the loss of acetylcholine synthesis.

4. In the 'warm' crop and gizzard, potassium produces contraction which is enhanced by eserine, but not abolished by nicotine or by atropine. With higher doses of potassium, stimulation is followed by inhibition. After short periods of cooling, the motor response to potassium is lost, but the inhibitory effect is still present. Prolonged cooling abolishes both actions. It is suggested that the augmentor action of potassium is due to an intermediate release of acetylcholine from the cholinergic nerve endings, and the inhibitory action to a liberation of adrenaline from the adrenergic nerves in the crop and gizzard.

5. Calcium inhibits the rhythmic activity of 'warm' preparations, and the effect of potassium. It has no action on cooled preparations, and in these it does not affect the contractions produced by acetylcholine. It is suggested that calcium acts on 'warm' preparations by preventing the release of acetylcholine from cholinergic nerve endings.

6. The action of adrenaline on 'warm' preparations is twofold: small doses have an augmentor effect; larger doses are inhibitory. After cooling, adrenaline has no action by itself. It is suggested that the augmentor effect of adrenaline is due to an improvement in acetylcholine-transmission at the cholinergic nerve endings.

7. Small doses of barium contract the 'warm' preparation. This action is inhibited by calcium, abolished by nicotine, and is lost after cooling. It is suggested that the action of such doses of barium is due to a stimulation of parasympathetic ganglion cells.

8. The presence of multipolar nerve cells in the enteric plexus was demonstrated in histological sections of the crop and gizzard. These were found lying between the circular and longitudinal muscle layers, in a position analogous to that of Auerbach's plexus.

We wish to express our gratitude to Prof. G. Payling Wright for his encouragement and for the loan of apparatus which made this work possible. We should also like to thank Messrs L. Oertling, Ltd., for the gift of the lever used in these experiments.

REFERENCES

Adolph, E. F. (1927). *J. Exp. Zool.* **47**, 31.
Bayliss, W. M. & Starling, E. H. (1899). *J. Physiol.* **24**, 99.
Bacq, Z. M. & Rosenblueth, A. (1934). *Amer. J. Physiol.* **108**, 46.
Beznàk, A. B. L. (1934). *J. Physiol.* **82**, 129.
Blaschko, H., Richter, D. & Schlossman, H. (1937). *Biochem. J.* **31**, 2187.
Brown, G. L. & Feldberg, W. (1936). *J. Physiol.* **86**, 290.
Bülbring, E. & Burn, J. H. (1939). *J. Physiol.* **97**, 250.
Chute, A. L., Feldberg, W. & Smyth, D. H. (1940). *Quart. J. Exp. Physiol.* **30**, 65.
Dale, H. H. & Gaddum, J. H. (1930). *J. Physiol.* **70**, 109.
Dikshit, R. B. (1938). *Quart. J. Exp. Physiol.* **28**, 243.
Feldberg, W. & Guimarais, J. A. (1936). *J. Physiol.* **86**, 306.
Feldberg, W. & Solandt, O. M. (1942). *J. Physiol.* **101**, 137.
Feldberg, W. & Vartiainen, A. (1934). *J. Physiol.* **83**, 103.
Feng, T. P. (1937). *Chin. J. Physiol.* **12**, 177.
Feng, T. P. & Dun, F. T. (1940). *Chin. J. Physiol.* **15**, 433.
Feng, T. P. & Shen, S. C. (1937). *Chin. J. Physiol.* **11**, 51
Gaskell, J. F. (1914). *Philos. Trans.* B, **205**, 153.
Hess, W. N. (1925). *J. Morph.* **40**, 255.
Hogben, L. T. (1925). *Quart. J. Exp. Physiol.* **15**, 263.
Lankester, E. R. (1865). *Quart. J. Micr. Sci.* N.S. **3**, 99.
Millott, N. (1943). *Proc. Roy. Soc.* B, **131**, 271, 362.
Mines, G. R. (1907). *J. Physiol.* **35**, xxiii.
Quastel, J. H., Tennenbaum, M. & Wheatley, A. H. M. (1936). *Biochem. J.* **30**, 1668.
Stephenson, J. (1930). *The Oligochaeta.* Oxford.
Vogt, M. (1943). *J. Physiol.* **102**, 170.
Wells, G. P. (1937). *J. Exp. Biol.* **14**, 117.
Wu, K. S. (1939). *J. Exp. Biol.* **16**, 184.

THE MECHANISM OF LOCOMOTION IN GASTROPOD MOLLUSCS

I. KINEMATICS

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(With Plate 1 and Eleven Text-figures)

Whereas the broad outline of the mechanical events of locomotion can in most animals be readily visualized, the mechanism of propulsion in gastropods is less easily observed. Since the seventeenth century (Lister, 1694) a great deal of attention has been paid to this problem and a wealth of suggestions has been forthcoming; there is, however, a significant absence of convincing experimental data. To some extent the problem is confusing on account of the diversity of locomotory mechanisms existing within the group (Vlès, 1907; Parker, 1911; Olmsted, 1917; Weber, 1925). Nevertheless, there can be no room for doubt that one characteristic type of movement depends upon the propagation of waves of muscular activity over the surface of the animal's foot. So far as is known, no adequate account has yet been given of the method whereby this type of activity propels the animal over the surface of the ground. The present paper deals with these problems in respect to three representative genera, *Helix*, *Haliotis* and *Pomatias*.

HELIX

Typically, when a snail (*Helix aspersa* or *H. pomatia*) begins to move, a dark transverse band appears near the anterior end of the under-surface of the foot; this band at once starts to move forward, but at the same time other bands appear posteriorly to the first until the whole surface of the foot (apart from a narrow lateral margin) is characterized by a pattern of forwardly moving bands. It is important to notice that the formation of the banded pattern begins at the anterior and proceeds to the posterior end of the foot at a speed considerably greater than the velocity with which the bands themselves move anteriorly over the foot; when these bands disappear the animal ceases to move. It seems generally agreed that the forward movement of alternating light and dark bands is due to the passage of co-ordinated waves of muscular contraction and relaxation. Each section of the foot in turn contracts and relaxes and in so doing changes its position forwards or backwards relatively to the rest of the body of the animal. Beyond this there seems no general agreement concerning the nature of these muscular waves or concerning the displacement of the pedal surface relative to the ground. Similarly, it has been suspected for some years that the passage of a muscular wave over a particular region of the foot involves vertical as well as horizontal displacement of the pedal surface. Experimental approach to this problem has, however, yielded two opposing views. Van Rijnberk (1919) and ten Cate (1922), using a manometer, convinced themselves that the visual moving dark waves represent ridges or projections from the surface of the foot; Olmsted (1917), working on various marine gastropods with similar technique, concluded that the areas in question represented furrows or concavities on the pedal surface; the latter conclusion was supported by Bonse (1935) whose technique was based on the displacement of the end of a small metal rod in contact with the surface of the foot. Whilst it is not claimed that the following observations resolve all questions dealing with the kinematics of the snail's movement, they nevertheless clarify the position to some extent.

Horizontal displacement of the pedal surface

Close observation of a snail moving over a glass plate shows clearly the forward movement relative to the ground of any point on the foot over which a dark band is passing, whilst the same point is at rest when lying within the wider and lighter areas. Biedermann (1905), however, claimed that a point on the surface of the foot is continuously in motion, moving at a slower speed when lying within a light area, and at a higher speed when within one of the darker bands. Bonse (1935), on the other hand, states that although movement only occurs during the passage of a dark band, nevertheless, this movement is itself discontinuous.

In order to obtain accurate information concerning horizontal movements, a number of points were defined on the surface of the foot by injection of small quantities of Indian ink or by means of an indelible pencil; the snail was then allowed to move over the surface of a vertical glass plate and cinematograph pictures (8–20 per sec.) were taken of the ventral surface. For more detailed observation of a localized area of the foot identification of fixed points is possible by noting the position of one or more of the relatively conspicuous mucous glands of the foot; these are readily identifiable

under the higher magnification employed and, therefore, form admirable landmarks.

When cinematograph records of this type are projected—or indeed when individual landmarks are observed by the naked eye—it can be noted that each landmark remains more or less stationary on the ground until one of the dark moving bands approaches it from a posterior direction; at this moment, the landmark begins to move forward with increasing velocity, but at a rate which is always less than that at which the wave itself is passing over the foot. As the centre of the wave passes the landmark, the velocity of movement of the latter falls until it finally comes to rest until the approach of the next

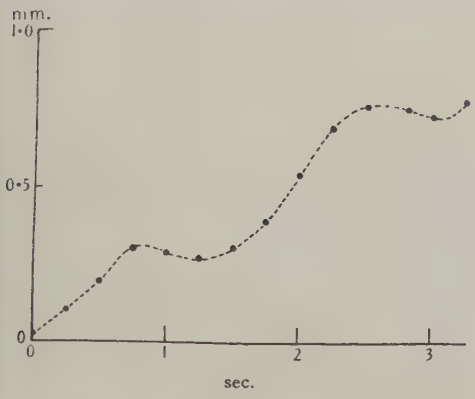


Fig. 1 *a*.

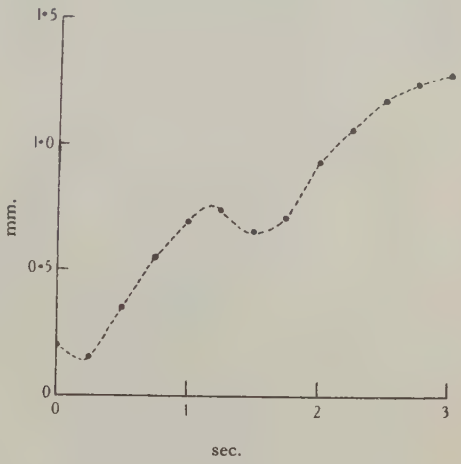


Fig. 1 *b*.

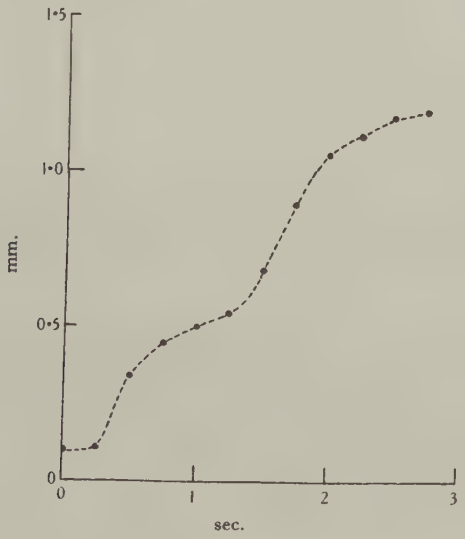


Fig. 1 *c*.

Fig. 1. Three types of forward motion of the mucous glands on the sole of *Helix pomatia*, as recorded by cinematography. The animal was moving vertically upwards. (*a*) represents the normal type of forward motion; (*b*) shows a certain amount of slip; (*c*) shows a passive forward drag in the relaxed state.

wave. The detailed analysis of the cinematograph records obtained from animals moving vertically upwards confirms this visual picture, but indicates a certain degree of variability in minor respects. The most common type of movement exhibited by a point on the surface of the foot is shown in Fig. 1*a* in which each period of forward movement is followed and preceded by a period of rest; the records show, however, that the periods of rest are definitely shorter than the time which elapses between the passage of two successive dark bands. In other words, the period during which a point is in motion has also been observed at or near regions which have been subjected to strong stimuli and in consequence show tonic contraction. It is therefore necessary to allow some time to elapse before records are made from landmarks made by the injection of Indian ink.

In order to correlate the movement of individual points with the functional state of their underlying muscles it is necessary to observe the movements of two adjacent points relative to each other and to the ground. An analysis of this type, derived from cinematograph records, is shown in Fig. 2*a*. In this figure the positions of three points *A*, *B*, and *C*

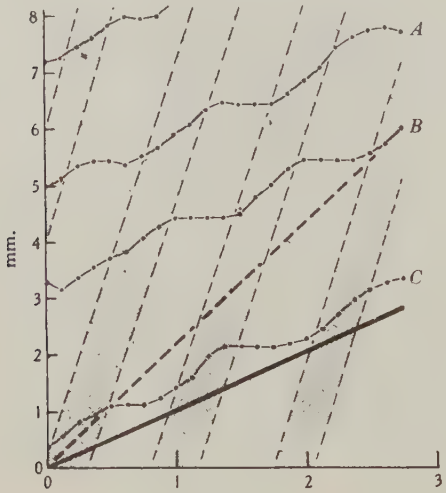


Fig. 2*a*.

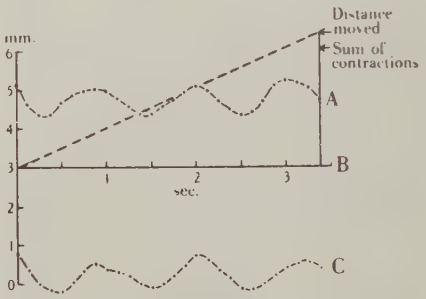


Fig. 2*b*.

Fig. 2. *a*. Graphs obtained from cinematograph records of the forward motion of three marked points arranged in antero-posterior direction on the sole of *Helix pomatia*. Full heavy line indicates the speed of the animal. Heavy dotted line the average speed of a point in forward motion. The shaded areas indicate the passage of waves of contraction. *b*. Analysis of the forward movement shown in *a*. The rate and degree of contraction of the distance *AB* and *BC* relative to point *B* is represented. The sum of contractions of *AB* approximately corresponds to the distance moved by the animal within that period.

is not restricted to the period of passage of the visual dark wave (see below). Other records (Fig. 1*b*) show, however, that there is often a definite backward movement at the completion of each phase of forward movement—in other words backward slip occurs: this phenomenon is particularly noticeable in the case of points lying near the anterior part of the foot or when the snail is made to creep a number of times over a track which is covered with the mucus deposited by previous movements, or when it was loaded. In the case of regions lying near the posterior end of the foot, landmarks often show continuous forward motion (Fig. 1*c*) indicating that they are kept in motion by energy derived from regions lying more anteriorly: this type of motion relative to the ground are shown on the ordinate, whilst time is plotted along the abscissa. The figure also shows the form, position and velocity of the waves (also plotted to scale from the photographs). It will be noticed that the distances between the two points *A* and *B* alternately increase and decrease; they are nearest together when they lie within the dark bands and farthest away when they lie at rest between two successive bands. This fact is shown very clearly in Fig. 2*b*. These results show conclusively that the dark bands seen on the pedal surface are essentially regions of longitudinal muscle contraction, whereas the lighter bands are regions of longitudinal relaxation.

So long as there is no slip between the ground and

areas of complete relaxation (as in Figs. 1*a*, and 2*a*) there must be a definite relationship between the forward velocity of the animal and the form and frequency of the waves of muscular contraction. The cycle of contraction and relaxation of each muscle fibre, shown diagrammatically in Fig. 3, is such that the duration of relaxation is twice that of contraction, and consequently for every fibre which is contracted there are two which are relaxed, and during the passage of one complete wave each of three fibres

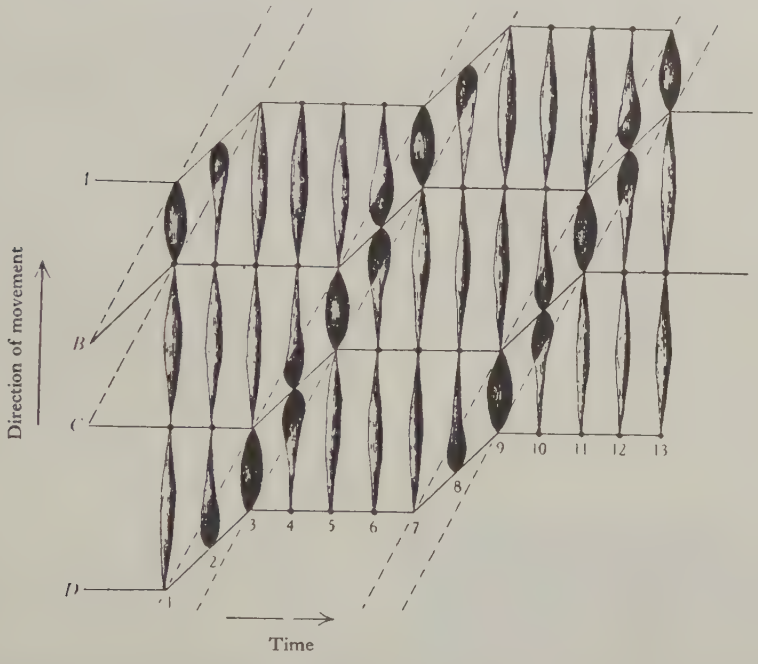


Fig. 3. Diagram correlating forward motion with the state of the muscles of the snail's foot. The position of the waves of contraction is indicated by the dotted lines. The muscle is assumed to contract to one-half of its original length, and it is separated from the succeeding wave of contraction by twice the distance of its own fully relaxed state. The speed of the animal resulting from such contractions and elongations is equal to the degree of shortening × frequency of contractions.

contracts once and each point on the pedal surface moves forward a distance which represents the difference between the length of full relaxation and contraction, provided that during contraction no significant vertical displacements accompany longitudinal movement.

Vertical displacements of the pedal surface

In the case of *Helix*, vertical displacements of the pedal surface are undoubtedly relatively small, but an attempt to define their nature was made as follows. A hole 2 mm. in diameter was cut in a glass or celluloid plate and through this hole passed the rounded end of a small glass recording lever; any vertical displacement of the pedal surface above or below the surface of the plate could thus be recorded (Fig. 4*c*). By observing and marking the passage of the muscular waves by means of a tapping key it was found that an upward movement of the lever coincided with the passage of the dark phases of the muscular waves. It has been claimed, however (ten Cate, Bonse), that marking with a tapping key introduces an error due to the reaction time of the observer. An attempt was made to avoid this complication by combining the record of the vertical displacement (Fig. 4*c*) with a simultaneous record of the horizontal displacement (Fig. 4*b*). Since the end of the lever in contact with the surface of the foot is dragged in the direction of the animal's movement, it may be assumed that any displacement thus recorded will have a value varying directly with the speed of an area gliding over it, i.e. it would correspond to the lowest point of a tracing as indicated in Fig. 4*b*. As the speed of this particular section of the sole decreases and finally comes to rest, the lever tends to swing back under its own

weight to its normal position, corresponding to the highest position in the tracing. A record thus obtained is given in Fig. 5.

From the above data it is possible to reconstruct the path of movement of any section of the pedal surface throughout one complete cycle of its muscular activities. Such a reconstruction can be followed graphically from Fig. 6. It will be noted

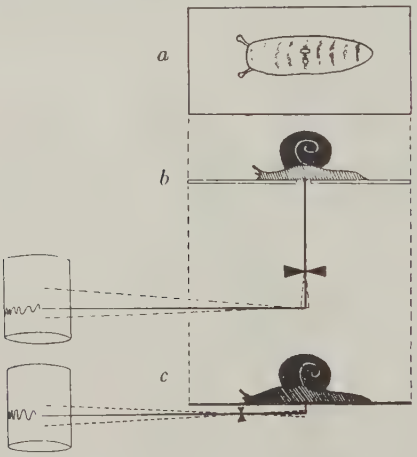


Fig. 4. Arrangement for simultaneous recording of horizontal and vertical displacements of the snail's foot during locomotion.

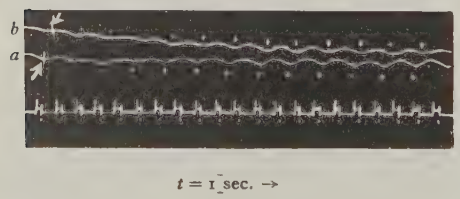


Fig. 5. Tracing obtained through the method illustrated in Fig. 4. *a*, horizontal; *b*, vertical displacement. The marks of coincidence indicate the position of the dark waves on the foot of the moving snail. These areas are lifted and move forward.

that as a wave of contraction and relaxation passes over any section on the pedal surface, the section in question is first moved upwards and forward, but later, whilst still travelling forward, begins to descend again to the surface of the substratum, where it comes to rest and exerts a downward pressure against the ground.

Taking the segment *F* in Fig. 6*a* it will be noted that its posterior edge lifts first and is the first to be placed on the ground in its new position of rest. In other words, the whole segment acts somewhat as the foot of a plantigrade vertebrate—the heel is lifted before the toes, and is also placed on the ground before the toes. It should be borne in mind that in Fig. 6 the vertical displacement of the segment is relatively very much greater than is actually the case in *Helix*. It is, however, convenient to emphasize this vertical movement in view of the phenomena to be described below for *Pomatias*.

HALIOTIS TUBERCULATA

Like *Helix*, *Haliotis* moves by means of a system of direct muscular waves. It is a very active creeper, with agile turning movements, and in some ways presents a clearer mechanical picture than the snail.

In *Haliotis* the locomotory waves are ditaxic in the sense that they alternate on the two sides of the foot; the form and frequency of these waves are highly characteristic. At any one time one complete and two partial, anteriorly moving, areas of somewhat darker appearance are visible on the sole of the foot (Fig. 7). Whenever one of these dark waves has reached the middle of the left half of the foot, the right side of the foot exhibits, at its anterior end, the posterior border of a dark area just fading out, whilst simultaneously another one is beginning to form at the hind end of the right half of the foot. As in *Helix*, the dark areas represent regions of maximum longitudinal contraction. These are most conspicuous along the outer lateral margin of the foot and seem to increase in length somewhat as they approach the anterior end; the zone of contraction, however, extends both in front and behind the dark areas.

The general co-ordination of movement relative to the body of a number of points along both halves of the foot is represented in Fig. 8, which is the analysis of a cinematograph record of *Haliotis* moving at a speed of 1 cm./sec. This shows: (i) the rhythm of contraction and relaxation of each section of the foot; (ii) the strict alternation of waves on the right and left half of the foot; (iii) two points on the sole, which are about 3·5 cm. apart when the intermediate zone is fully relaxed, approach each other to about 2 cm. when the intermediate zone is fully contracted (length of foot 5·5 cm.). As in *Helix* the degree of contraction, in this case 1·5 cm., makes up the total distance of one 'step' relative to the ground, and obviously the length of a step × frequency of steps (waves) equals the speed of the animal relative to the ground.

Each of the marked points on the sole of *Haliotis* moves forward relative to the ground in essentially the same manner as they do in *Helix*, though in *Helix* every wave of contraction carries the point forward by 0·3–1·2 mm., while in *Haliotis* the corresponding distance amounts to between 1·5 and 2·3 cm. (Fig. 9). This condition is true for any point along the lateral part of the foot, where there

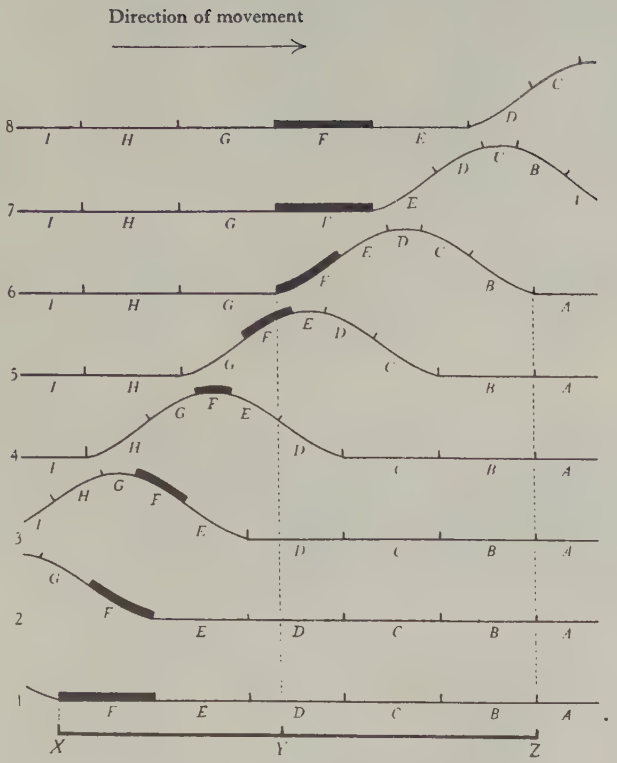


Fig. 6*a*. Lateral view.

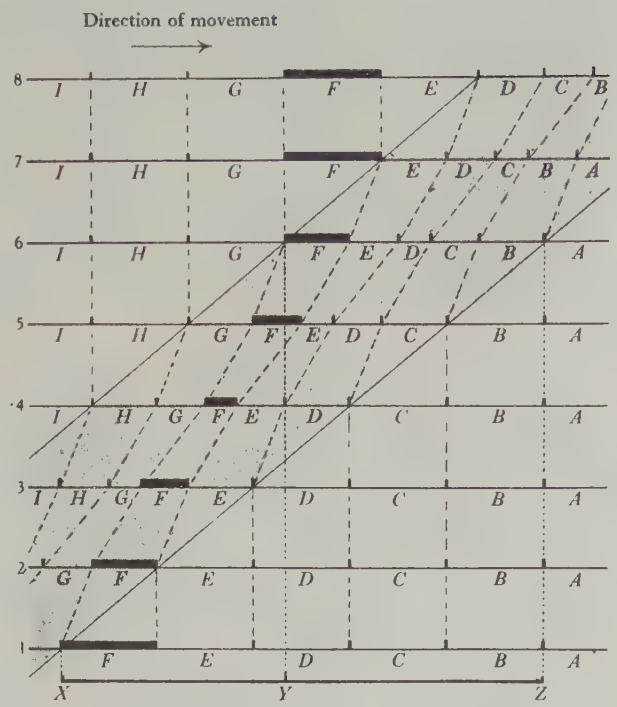


Fig. 6*b*. Ventral view.

For descriptions of Figs. 6*a* and 6*b* see p. 64.

is a regular alternation of forward motion and rest. Fig. 9 suggests that, as in *Helix*, three different types of forward motion can occur: Towards the anterior region of the animal a minute backward slip can be observed after the phase of forward motion; this is normally absent towards the middle of each lateral area of the foot, whereas at the posterior end the sole occasionally fails to engage the ground and is passively drawn forward in a relaxed state.

come apparent from a shortening of a distance between two points in the contracted region, or from a change of its optical properties, but also from a lateral deformation of the foot. At the outer lateral margin an outwardly directed bulge accompanies the passage of each longitudinal contraction, and any point at the same level on the median line is pushed in the opposite direction. As a result of the functional alternation on the two halves of the foot

Fig. 7. Locomotion of *Haliotis tuberculata* (redrawn from film) showing the passage of darker areas and lateral deformations of the foot. The foot was marked at two places on the right half and at one on the left. ● indicates that the marked point is stationary, and ○ indicates movement relative to the ground. The series reads from left to right, and the figures give the time in $\frac{1}{16}$ sec. The transverse lines mark distances of 1 cm.

This picture, however, applies only to the outer lateral margin of the foot, for in *Haliotis* there is no alternation of relaxation and contraction along the median line. Any two points on this line always keep the same distance apart, and move forward at a more or less uniform speed while the animal is in motion (Figs. 9, 10). The underlying muscular tissue cannot be regarded as active in any locomotory sense.

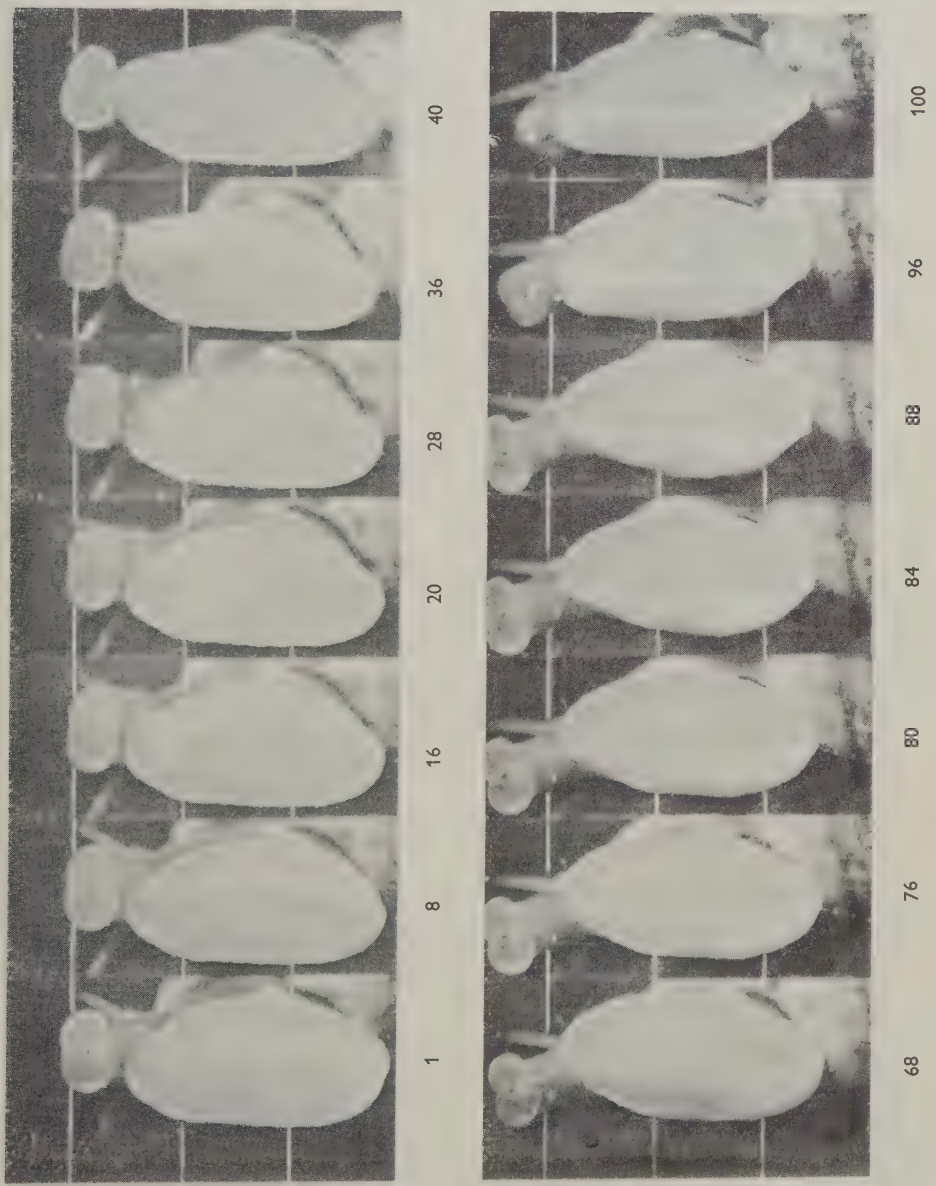
As can be seen from Fig. 7, in *Haliotis* the contraction of longitudinal muscles does not only be-

any point on the median line, though moving forward at a constant speed, performs at the same time lateral movements of the same frequency as the locomotory waves (Fig. 10). At the present state of our knowledge, it appears uncertain whether this lateral displacement may be taken as being indicative of an antagonism between longitudinal and transverse fibres in the sole of the foot, as assumed by Vlès (1908), or merely as resulting from a thickening of the contracted longitudinal fibres.

It is worth noting that in *Haliotis* the lifting of the

Legend to Text-figures 6*a* and 6*b*.

Fig. 6. Diagram illustrating in 8 successive positions the movement of a section of the snail's foot. *a*, lateral aspect; *b*, ventral aspect. The fully relaxed sections of the foot are fixed to the ground. A complete wave of contraction involves at any one time five adjacent sections, e.g. *B–F* in position 6, showing a varying degree of shortening. The ratio of full relaxation to full contraction of any one section is taken to be 3:1. The phase of contraction is associated with simultaneous vertical and horizontal displacements of the pedal surface. As represented in the diagram, any one point completes its phase of forward motion after 6 successive positions. The horizontal distance travelled by any one point (*XY* for the posterior edge of section *F*) equals the difference in length of all sections involved simultaneously in contraction (*YZ* for the sections *BCDEF* in position 6) and the length of all these segments in a state of full relaxation (*XZ* in position 1). Therefore: the length of one step, for the posterior edge of section *F*, $xy = xz - yz$. Obviously the speed of the animal is equal to the length of a step × frequency of the waves.



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contracted parts is clearly observable, and it sometimes gives the appearance as if the surface of the sole is thrown into folds through longitudinal contractions. Furthermore, as the phase of contraction progresses forward the anterior border of the lateral bulge (Fig. 7) becomes irregular in outline; this phenomenon may be due to the detachment by the action of the longitudinal fibres of regions of the sole which are adhering to the ground.

and left halves being separated by a distinct median line. Movement is initiated by a slight swelling of one half of the foot which expresses itself as a lateral and medial expansion (Fig. 11); it is accompanied by a corresponding decrease of the surface area of the adhering surface of the other half. While this process is still in progress, a wave of contraction passes over the smaller half, causing it to be lifted off the ground and to be shortened longitudinally. The wave quite

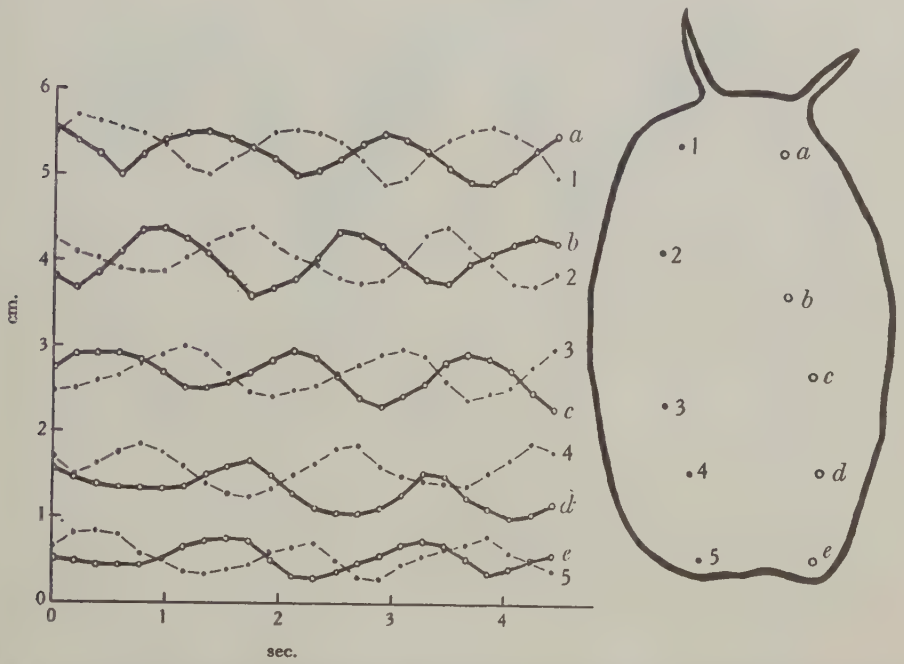

Fig. 8. Co-ordination of movement relative to the body of 10 points on the sole of *Haliotis tuberculata* moving at a rate of 1 cm./sec.; *a* and 1 shew the position of the anterior margin of the foot, *e* and 5 the posterior margin. Note the strict alternation of the right and left halves of the foot, and that the sections *a*–*c*, *c*–*e*, 1–3, 3–5 show the absolute maximum degree of shortening, which in this case amounts to about 1·5 cm.

POMATIAS ELEGANS

In 1882 Simroth gave a general account of the locomotory movements of a specimen of *Pomatias elegans*. Unfortunately his description and interpretation are closely linked up with his theory of active muscular extension, and they cannot be confirmed in a number of essential details; he, and earlier observers, agree on the difficulty of observing this rather shy species.

The following description is based on a film analysis of animals moving on a vertical glass plate (Pl. 1). When the animal is at rest, the foot is approximately symmetrical about the median line, the right

obviously starts from the hind end, first detaching the posterior and outer lateral margin, and running obliquely forward, the region near the median line being detached somewhat later. This phase of contraction involves the entire length of one half of the foot, which for some short period is completely lifted off the ground. No sign of waves passing over the raised half of the foot can be discerned on the photographs, nor have any such waves been noticed on the living animals—contrary to Simroth's description.

The stationary half foot swells up until the other half is lifted, but the lateral extension of its sole begins to decrease as soon as relaxation sets in in the

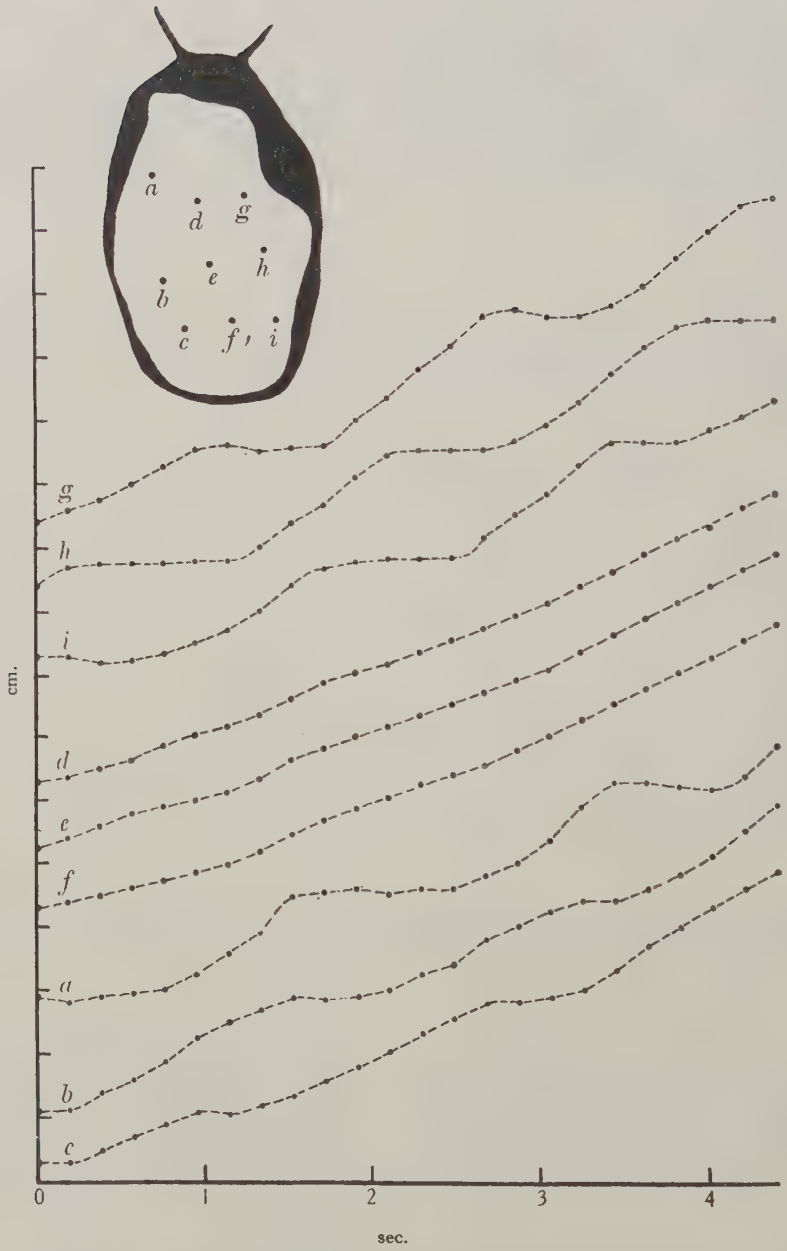

Fig. 9. Movement relative to the ground of nine points on the sole of *Haliotis tuberculata*. Note that the phase of forward motion and rest, which can be seen on the laterally situated points, are more clearly differentiate in the anterior region. The points along the median line progress at a constant speed. In the outline figure th initial positions of the points are marked.

lifted half. This relaxation causes the posterior border of the relaxing half foot to be brought down on to the substratum first, thereby obviously establishing a posterior *point d'appui* against which the anteriorly situated regions spread forward as relaxation proceeds in the same direction. Ultimately this half comes to rest in a more anterior position relative to the foot which remained stationary. When both halves are thus again completely placed on the ground, there is a considerable interval during which no forward movement occurs. It can be noticed, however, that during this period the conditions for the next step are being prepared by a reversed swelling and de-swelling of the two half feet (Fig. 11).

After the initial short step, each subsequent longitudinal contraction moves the contracting half foot anteriorly to a position level with the stationary foot, and each succeeding relaxation causes it to move to a position more anterior than that which is occupied by the stationary foot, i.e. the total movement is twice the distance of the initial short step.

A lifting of the contracted half has never been observed to begin from the median line, as stated by Simroth, nor has any instance been recorded when the foot was put down with the anterior edge touching the ground first. Occasionally, in less active animals, the more medially situated regions of the foot are not raised at all, but in every case both the raising and the lowering, which coincide with longitudinal contraction and relaxation, start from the hind end and spread forward.

In *Pomatias* the proboscis plays a part in locomotion. It can be seen to attach itself after being pushed forward, and naturally it must be detached from time to time as the foot advances. Its activity does not seem to be rigidly correlated with the rhythmic alternation of pedal movements. Pending further investigations it is suggested that its activity is mainly governed by the tone of the columellar muscle.

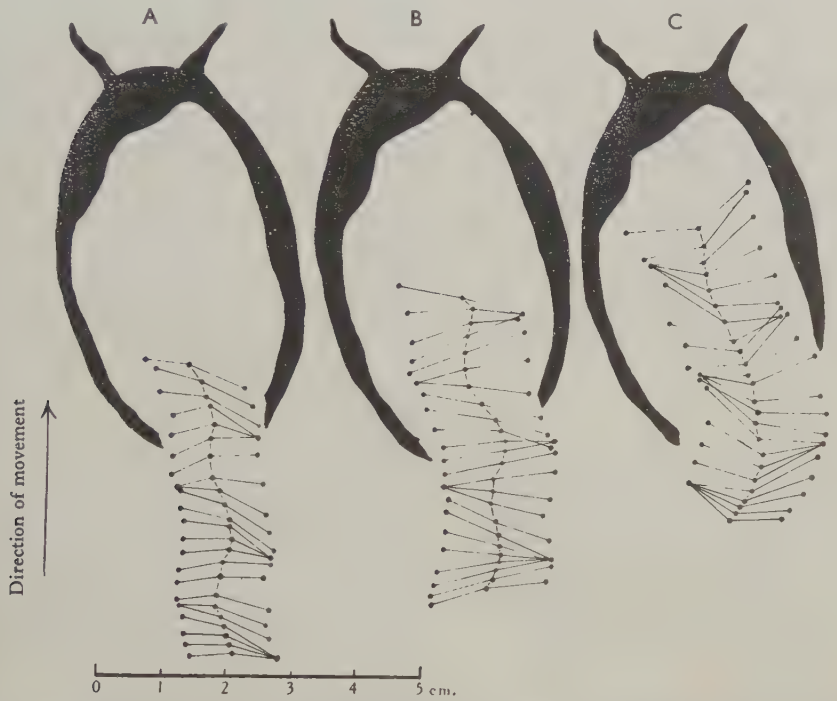

Fig. 10. Successive positions relative to the ground and to each other of nine points on the sole of *Haliotis tuberculata* in locomotion, showing a lateral movement of the median line. In *A* the points marked lie near the posterior end of the animal, in *B* the points are situated midway along the foot, and in *C* they lie further towards the anterior end. The transverse lines connecting the points indicate coincident positions. The lowest of the transverse lines in each of the three diagrams shows the initial position relative to the ground of the three points marked on the sole; the uppermost transverse line shows the points when the animal has reached the position indicated by the outline. Note that the ground is more effectively engaged by the points lying at the anterior end of the animal than by the points lying further behind.

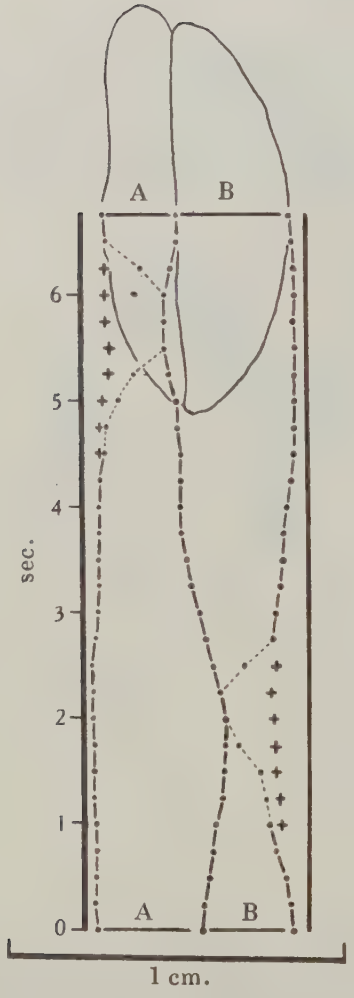

Fig. 11. Changes in the lateral extensions of the right (A) and left (B) half of the foot of *Pomatias elegans* in locomotion (ventral aspect). + indicates the position when the lateral margin is lifted off the ground. The width of the stationary half of the foot increases medially and laterally until the other half is lifted. The outline of the foot indicates the final position reached.

CONCLUSIONS

The diversity of locomotory types—ranging from undulations of a peristaltic nature to alternate stepping—encountered here within a group of relatively close phyletic coherence such as the gastropods, recalls similar conditions in other groups, viz. arthropods, vertebrates, etc.

The movement of the two halves of the foot of *Pomatias elegans* can best be compared with the ambulation of a bipedal vertebrate. The heel is lifted first, then the toes; the heel is put down first with the toes following. In *Helix*, on the other hand, the conditions are more comparable to a millipede or a caterpillar, although here again the movement of each localized area of the foot is comparable to that of the plantigrade surface of a tetrapod limb. *Haliotis* occupies an intermediate position between *Helix* and *Pomatias*. The diagonal co-ordination of movement of the sole of *Haliotis*, with three simultaneous areas of motion separated by three areas of support, is clearly reminiscent of conditions found in hexapods, and offers some obvious advantages with regard to stability (Gray, 1944) and turning movements. In this connexion it appears significant that, according to Robert (1907), in the similarly moving *Trochocochlea* the locomotory waves can be reversed on one side when a turning movement is executed. It is clear that the difference between *Helix*, *Haliotis*, and *Pomatias* consists merely in a difference of wave-length relative to the total length of the locomotory surface, in that longer series of muscle fibres are grouped together into one continuous region of contraction. When this process has gone so far as in *Pomatias*, and involves the whole length of the foot, the functional alternation of the two halves of the foot becomes an absolute mechanical necessity.

It must be borne in mind that the three genera discussed here represent but a small selection of modifications in locomotory methods found amongst gastropods. However, while it is impossible at the present state of our knowledge to offer a plausible suggestion as to why such a great functional variety has arisen, the conditions appear suggestive enough to look for an explanation along similar lines as has been advocated for vertebrates, where, in the course of evolutionary changes, land-living tetrapods have emerged from an undulating aquatic type. From a physiological point of view the locomotory waves of *Helix* are usually classed with undulations as seen to pass over the body of a moving fish or annelid worm (peristalsis). It is, however, important to remember that the locomotory waves in *Helix* cannot, for mechanical reasons, represent the primary waves of locomotion of an organism originally swimming through water, because their direction would tend to drive the animal backwards. It seems more profitable to take into account the facts which point to a turbellarian resemblance of gastropods. So far as locomotory types are concerned, a great number of parallels can be found existing in both groups: (i) ciliary locomotion, common in Turbellaria and occurring in some gastropods (Copeland, 1919, 1922; Gersch, 1934; Olmsted, 1917); (ii) swimming through parapodial movement as seen in *Thysanozoon* and *Aplysia*; and above all (iii) muscular locomotion for sliding over more or less solid surfaces.

This latter mode shows a great variety of co-ordination in gastropods, and it is interesting to note that an alternation of the locomotory activity of the right and left halves is found in certain marine Polyclades (Olmsted, 1922). This type of creeping locomotion differs, however, in some respects fundamentally from the type of locomotion exhibited by an annelid worm (Gray & Lissmann, 1938): (i) in that the areas of fixation are wide and represented by zones of longitudinal muscular relaxation; (ii) the parts in motion are short and longitudinally contracted; (iii) the propagation of waves takes place in postero-anterior direction. The significance of these facts could perhaps be more fully appreciated if the forces set up during one complete locomotory cycle were better known.

SUMMARY

1. The modes of progression of *Helix*, *Haliotis*, and *Pomatias* are described.

2. In all three species waves of longitudinal muscular contraction, followed by relaxation, pass over the foot in postero-anterior direction.

3. Typically, any point on the sole shows during one locomotory cycle a phase of forward movement and a phase of rest. Minor variations depend on external conditions, and on the location of such a point on the sole.

4. In all three species the longitudinally contracted areas are lifted off the ground and move forward, whilst the elongated areas remain stationary.

5. The length of a step is essentially determined by the difference between the length of the musculature comprising the contraction phase of one locomotory wave, and the length of this musculature when it is fully relaxed (Fig. 6).

6. The difference between the three species consists in a difference of wave length relative to the total length of the foot. In *Haliotis* and *Pomatias* there is a functional alternation of locomotory phases of the right and left halves of the foot.

I am greatly indebted to Prof. J. Gray, F.R.S., for his interest and help in the course of these investigations. Part of the work was done while holding the Cambridge University Table and a grant from the Bidder Fund at the Stazione Zoologica in Naples. I wish to express my thanks to the Director and staff for the facilities they gave me.

REFERENCES

Biedermann, W. (1905). *Pflüg. Arch. ges. Physiol.* **107**, 1.
Bonse, H. (1935). *Zool. Jb.* **54**, 349.
Copeland, M. (1919). *Biol. Bull. Woods Hole*, **37**, 126.
Copeland, M. (1922). *Biol. Bull. Woods Hole*, **42**, 132.
Gersch, M. (1934). *Biol. Zbl.* **54**, 511.
Gray, J. (1944). *J. Exp. Biol.* **20**, 88.
Gray, J. & Lissmann, H. W. (1938). *J. Exp. Biol.* **15**, 506.
Lister, M. (1694). *Exercitatio Anatomica in qua de Cochleis maxime terrestribus et Limacibus agitur*. London.
Olmsted, J. M. D. (1917). *J. Exp. Zool.* **24**, 223.
Olmsted, J. M. D. (1922). *J. Exp. Zool.* **36**, 57.
Parker, G. H. (1911). *J. Morph.* **22**, 155.
van Rijnberk, G. A. (1919). *Arch. néerl. Physiol.* **3**, 539.
Robert, A. (1907). *Bull. Soc. zool. Fr.* **32**, 55.
Simroth, H. (1882). *Z. wiss. Zool.* **36**, 1.
ten Cate, J. (1922). *Arch. néerl. Physiol.* **7**, 103.
Vlès, F. (1907). *C.R. Acad. Sci., Paris*, **145**, 276.
Vlès, F. (1908). *Bull. Soc. zool. Fr.* **33**, 170.
Weber, H. (1925). *Z. vergl. Physiol.* **3**, 389.

EXPLANATION OF PLATE 1

Successive cinema-photographs showing the ambulation of *Pomatias elegans*. The transverse lines indicate a distance of 5 mm., the figures the time in $\frac{1}{16}$ sec. Note that the contraction starts at the hind end and spreads anteriorly. At 28 it involves the whole length of the left half, and at 88 of the right half of the foot, which at these stages are lifted off the ground and brought from a more posterior position to the level of the stationary relaxed half of the foot. Relaxation sets in at the posterior end (36 and 96); the foot is first lowered at the posterior edge, thereby establishing a posterior *point d'appui* against which the anteriorly situated parts move forward as relaxation and elongation proceeds. In the long interval between 40 and 68 no forward motion occurs, but a very significant change in the adhering surface area of the two halves is noticeable which takes place in preparation for the next step.

INTERNAL OXYGEN ENVIRONMENT OF THE BRAINS OF POST-MATURE RABBIT EMBRYOS

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(With Three Text-figures)

In 1935 Barcroft, Flexner, Herkel, McCarthy & McClurkin, working on rabbits, showed that the oxygen saturation of the blood leaving the uterus dropped from an average value of about 70% at the 18th day of gestation to about 30% saturation on the 30th day. During the period in question the foetuses are in active growth, whilst growth of the placenta is only trifling. On the 18th day the combined weight of the foetuses is approximately the same as that of the foetal portions of the placentas to which they are attached; on the 30th day the foetuses weigh about fifteen times as much as the placentas.

The alterations in weight taken together with those in the saturation of the blood suggest that the provision for the oxygen supply of the foetus is more or less fixed about two-thirds of the way through pregnancy in the rabbit, and that at the end of the gestation period the embryo has exploited that provision to the full: in short, that its metabolism has reached a limit where the foetus must either be born or stop growing.

Snyder (1934) had, however, shown that by the injection of prolan into the mother on the 25th day not only could the period of gestation be prolonged by several days, but that the foetuses continued to grow. It is difficult to give exact comparisons as regards weight, because of the diverse breeds of rabbit used in ordinary laboratory experiments—apart from the observations of Hammond, who has worked on carefully selected strains. Moreover, the weight of each individual rabbit foetus depends to some extent on the total number of young in the litter; the more numerous the individuals, the smaller tends to be the weight of each. In general, however, the weight of a laboratory rabbit at birth (31 days) is about 50 g., whilst the post-mature prolan rabbits at 35 days have for the most part weighed 70–80 g., i.e. the 35-day rabbit is about half as large again as the normal rabbit at birth.

The above facts raise the question: Can so great and rapid a growth take place with no more than the ordinary placental apparatus, stereotyped in weight at all events, by the time the prolan is injected? Does the placenta, as the result of the prolan, grow in proportion to the embryo? Or does the foetus eke an increasingly exiguous existence until at last at 36 days it dies of asphyxiation or inanition?

The word asphyxiation introduces the conception of oxygen want, a condition which it seemed possible to explore. Conditions of oxygen environment have a special importance on the ground of the construction which Rosenfeld & Snyder put upon observations which they subsequently made on post-mature foetal rabbits. These experiments were of the most careful kind, and the experimental facts are not in doubt; it is the interpretation of the facts which in our view demanded further exploration.

Snyder & Rosenfeld say, 'Oxygen want depresses or abolishes foetal respiratory movements'. This statement is based on the fact that administration of 'a gas of low oxygen content' to the mother, on and after the 32nd day, lowers the rate of rhythmic movement as seen through the uterine wall, or even abolishes the gasps altogether. In view of the fact that the maternal blood leaving the uterus at 30 days is already very greatly reduced, the question arises whether foetal rabbits at term are not in any case under conditions of anoxaemia. If they are, this condition is not likely to be improved, so far as the uterine circulation is concerned, by section of the spinal cord—the procedure which Snyder & Rosenfeld adopted to produce local anaesthesia.

It might, therefore, be that instead of making the general statement quoted above, Snyder & Rosenfeld might more properly have said, 'Oxygen want *beyond that to which the foetuses were already subjected* depresses or abolishes the foetal respiratory movements'. The research described below was undertaken to shed more light on the general condition of post-maturity in rabbit embryos, and particularly on the degree of anoxaemia to which they are subjected.

PRELIMINARY EXPERIMENTS

The first attempt made to study the conditions of oxygen exchange in post-mature foetuses was an extension of the method used by Barcroft *et al.* (1935), i.e. of analysing the blood leaving the uterus. The result showed no decrease in the oxygen

saturation of the venous blood further than had been observed during the normal period of pregnancy.

Rabbit 4509: urethane progesterol injected subcutaneously: weight 3·6 kg., four foetuses, average weight 58 g., age 33 days; arterial blood bright red; blood from left uterine vein 40·6 % saturated; foetuses quiescent.

In our next experiment we opened the abdomen and drew our samples of blood from three of the large veins draining the uterus. The most remarkable feature of these samples was the difference of oxygen saturation which they presented.

Rabbit 4689: urethane, 500 i.u. progesterol injected intravenously on the 28th day; foetal age 33 days; five foetuses, average weight 41·1 g.; foetal movements could be seen through abdominal wall, and through uterine wall when abdomen was opened; oxygen saturation of blood in veins draining uterus (1) 34 %, (2) 65 %, (3) 44 %.

In the course of this experiment we noticed that the blood reaching these vessels from the cotyledons appeared to be much darker than that reaching them from the muscle of the uterine wall. We therefore determined to transfer our attention to the foetus itself.

The most natural vessels to select in order to study the internal environment of the foetus in respect of oxygen would be the umbilical artery and vein. These, however, are short, not very accessible, and constrict on extremely little provocation, thus making it difficult to preserve the normal conditions of flow.

Having regard to the fact that we were more interested in the brain than in any other organ, we turned our attention to the technique used by Barcroft, Barron, Cowie & Forsham (1939), in which venous blood is withdrawn from the sinus at the junction of the frontal and parietal bones. This technique proved extremely simple and satisfactory.

METHODS

We are indebted to Mr John Hammond, Jr., for the preparation of the mothers, by injections of 100 i.u. chorionic gonadotrophine intravenously and 5 mg. progesterone subcutaneously on the 25th day of pregnancy.

OPERATION

The rabbit was given urethane 1 g. per kg. as a 25 % solution injected into the ear vein. It was then placed in a saline bath at 39–40° C. The abdomen was opened in the middle line. In general a portion of uterus can be seen in which there is a foetus. The size and further shape of the abdominal opening must be regulated by the way in which this portion of the uterus presents itself. There must be no undue 'pulling about' of the uterus, because it is vital that the relation of the foetus to its placenta should be unimpaired. The position of the head can be seen through the uterine wall, and a slit is made in the wall just over the crown of the head and along its longitudinal axis. The top of the skull is exposed by removal of the skin on the crown of the head. The point of a hypodermic needle, attached to a syringe, is inserted in the middle line into the anterior fontanelle. The point of the needle is then passed backwards in contact with the cranial wall till it reaches the posterior fontanelle; from there the blood is drawn.

Withdrawal of blood

The syringes best suited for the purpose are those the plungers of which are withdrawn by a screw mechanism. The blood can thus be collected gradually and at a more or less uniform speed. The dead spaces of the syringes have previously been filled with liquid paraffin; 0·2 c.c. of blood is taken.

The sample is at once transferred under paraffin to 0·5 c.c. of oxygen free oxalate-fluoride mixture, which overcomes its very great tendency to clot, and the oxygen content and capacity are measured in the van Slyke manometric apparatus.

For purposes of calculating the percentage saturation it has been our usual practice to determine the oxygen capacity on the sample of blood used for the content, either by the van Slyke apparatus, or by a Haldane haemoglobinometer calibrated with a 'van Slyke': any errors in dilution affect both the oxygen capacity and the oxygen content estimations equally. On the other hand, the haemoglobin determinations shown in Fig. 3 are mostly the result of separate observations on blood collected from the jugular vein and estimated with calibrated haemoglobinometers.

RESULTS

It is known from the work of Hammond (1935) on rabbits of uniform breed that after 29 days there is no exact correlation between the weight of the foetus and that of the placenta. This is true even within the same litter, and examples in Table 1 show that it is true of the injected rabbits no less than of the normal ones—see A_3, 32 days, where the heaviest placenta, 6·1 g., is attached to by no means the heaviest foetus. Though it is perhaps worth noting that the one really 'outsized' foetus in the series—93 g.—in a litter in which the other recorded foetuses weighed between 56 and 63 g., was attached to a placenta which weighed 8·6 g., the others weighing 4·4–3·7 g. Judging from the weights of injected and control rabbits observed on the 28th to 31st day, there is no reason to suppose that the injection either stimulated or stunted the growth of the foetuses.

The mean weights of the foetuses which we used in each litter, and the 'attached' foetal placentas, are given in Fig. 1. While on the whole the weight of the foetuses increases as intra-uterine life proceeds, and this applies to the period of post-maturity no less than to that of normal gestation, it is difficult to make

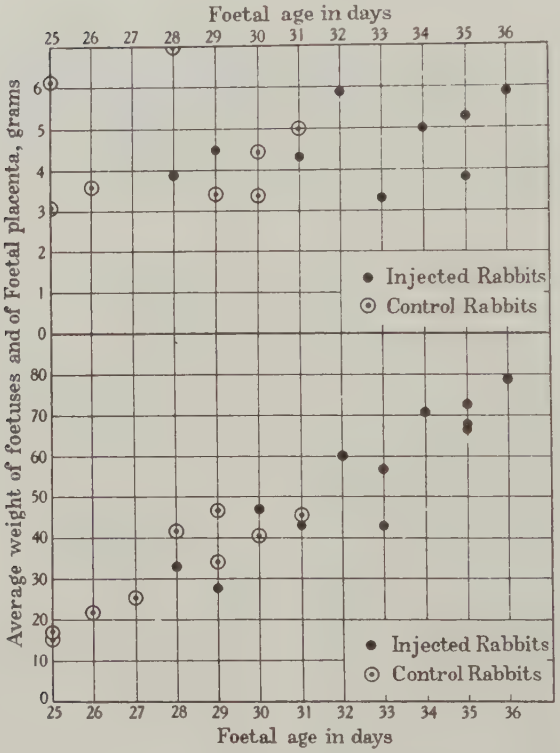

Fig. 1. Growth of foetuses and placentas respectively in injected rabbits and controls. Each point represents the average weight of the foetuses or placentas used from a single litter.

any corresponding statement about the weight of the placentas. The extremes at 25 and 35 days respectively are not very different from one another.

The oxygen in the blood collected from the posterior fontanellé

Table 2 gives the results of analyses of blood samples collected from the posterior fontanelles of rabbit foetuses between 25 and 35 days foetal age.

The determinations are shown in Fig. 2.* In Fig. 3 the arithmetical mean of the oxygen capacities of the foetuses used in each experiment are plotted against the foetal age.

DISCUSSION

In pregnant rabbits into which appropriate doses of chorionic gonadotrophin have been injected on the 25th day the foetus continued to grow, as was shown by Snyder. The increase from the 25th day onwards is roughly in a linear relation to their age. This means that the 'rate of growth' is becoming slower, otherwise the weight would increase in a geometrical progression. The percentage saturation of the blood obtained from the posterior fontanelle falls progressively from the 25th day onwards. That it should fall towards the end of pregnancy is in line with what is known to take place in the sheep foetus. At term (31 days) in the rabbit the saturation in the fontanelle is about 50%, which is higher than that in the sheep—about 20%. As showing the efficiency of the placental and foetal circulations, it is interesting to note that at term the blood from the fontanelle is about 50% saturated, whilst that from the uterine vein is only about 30% saturated (Barcroft, 1935). Yet, to reach the fontanelle from the mother, the oxygen has to diffuse through the placental barrier, and, after traversing the umbilical vein, to mix with

* It is right to say that one observation has been omitted from this figure on the ground of its inherent unlikelihood, 92% saturation in a 25-day rabbit; apart from gross contamination with air, which is believed not to have taken place, the rupture of an artery might produce such a result.

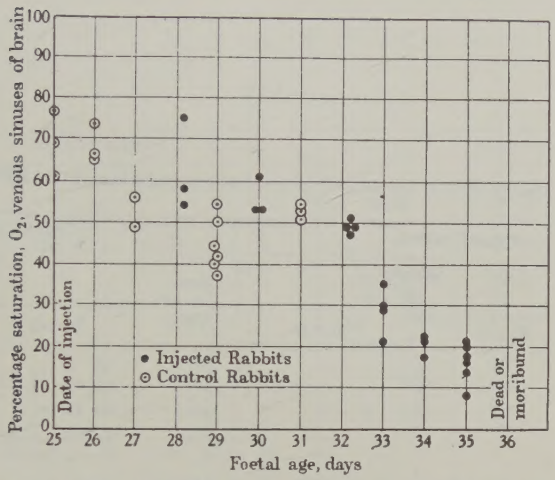

Fig. 2. Percentage saturation of blood from the anterior fontanelles of foetuses from injected rabbits and controls. Each point represents a single foetus.

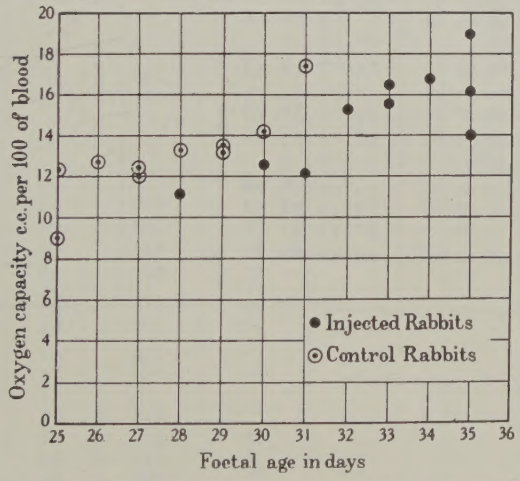

Fig. 3. Oxygen capacity of blood usually from jugular vein. Each point represents the average for the foetuses used for blood gas experiments in a single litter.

venous blood from the liver, there to evade the venous blood from the superior vena cava and azygos veins: traverse the heart, mix in the left side of the heart with blood coming from the foetal lung (which of course reached the lung as venous blood from the right side of the heart), traverse the carotid and finally the capillaries of the brain.

In the case of the post-mature foetuses the oxygen saturation in the fontanelle blood continues to drop as the days pass, until at the time beyond which it is impossible for the foetuses to live, the blood emerging from the brain is almost denuded of oxygen. It seems clear that placental development does not, as the result of prolan injection, take place *pari passu* with that of the foetus, and pending the exploration of other contributory causes of death, asphyxia is sufficient to be regarded as the probable cause of the cessation of life. Nor does it seem possible in the

Table 1. *Giving data of the weights of the foetuses and placentas at different ages*

Foetal age days	Injected rabbits			Control rabbits		
	Serial no. of rabbit	Weight g.		Serial no. of rabbit	Weight g.	
		Foetus	Placenta		Foetus	Placenta
25	—	—	—	5010	18 17	6·1 6·0
				5025	15 14 15 16	3·3 3·3 4·6 3·6
26	—	—	—	5026	20 22 23	2·8 3·2 3·0
27	—	—	—	5029	24 28 24 25	5·4 4·6 5·6 4·7
				A_5	27 25 25 25	— — — —
28	5008	32 31 36	4·1 3·5 3·7	5203	42 41 39	6·5 7·9 —
29	5036	24 31 28	3·9 4·3 5·4	5022	33 32 34 38	3·5 3·4 2·5 3·8
				5007	41 42 53 46	— — — —
30	5003	49 42 50	— — —	5034	42 40 45 33	3·9 4·8 3·8 5·1
31	5038	37 38 48 47	3·0 4·1 5·1 6·6	A_6	47 44 46 42	4·6 5·3 5·6 4·6
		39 42 46 40	— 4·9 6·1 3·8			
		48 44	4·0 5·1			
32	A_3	63 60 63 57	5·2 6·6 5·9 5·3			
33	A_2	56 62 59 48	— — — —			
	5021	40 50 36 44	3·3 3·1 3·0 3·9			
34	A_1	72 72 74 65	3·3 6·2 5·5 3·9			
35	A 4623	75 75 71	— — —			
	A_7	63 56 60 93	4·4 3·7 3·8 8·6			
	5033	63 59 76 65	3·8 4·4 3·8 3·1			
		61 71 76	3·0 4·2 5·1			
36	5009 (moribund)	95 78 42 101	4·4 5·4 6·0 6·1			

Table 2

Foetal age days	Injected rabbits				Control rabbits			
	Serial no.	Oxygen			Serial no.	Oxygen		
		Content c.c. %	Capacity c.c. %	Saturation %		Content c.c. %	Capacity c.c. %	Saturation %
25	—	—	—	—	5010	6·83	8·8	77
					5025 (1)	7·5	12·3	61
					(2)	8·6	12·4	69
26	—	—	—	—	5026 (1)	7·9	11·7	67
					(2)	8·7	12	73
					(3)	7·5	11·4	66
27	—	—	—	—	A_5 (1)	6·9	12·3	57
					(2)	5·6	11·4	48
28	5008 (1)	7·3	9·8	75				
	(2)	6·5	11·1	58				
	(3)	6·5	12·2	54				
29	—	—	—	—	5022 (1)	8·5	17	50
					(2)	9·6	17·6	55
					(3)	6·5	17·6	37
					(4)	7·8	18·5	42
					5007 (1)	5·6	14·1	40
					(2)	5·6	12·8	44
30	5003 (1)	7·1	13·5	53				
	(2)	7·7	12·4	62				
	(3)	6·5	12·2	53				
31	—	—	—	—	A_6 (1)	9·2	16·9	54
					(2)	8·9	17·4	51
					(3)	8·7	16·6	52
32	A_3 (1)	6·6	14·0	47				
	(2)	6·6	13·0	51				
	(3)	7·1	14·7	48				
	(4)	7·3	15·1	48				
33	A_2 (1)	5·7	16·5	35				
	(2)	4·8	23	21				
	(3)	5·3	17·6	30				
	(4)	4·8	16·5	29				
34	A_1 (1)	2·9	17·1	17				
	(2)	3·7	16·8	22				
	(3)	3·1	—	—				
	(4)	3·4	16·2	21				
35	A4623	2·0	11·7	17				
		2·0	13·9	14				
	A_7	3·2	17·6	18				
		3·0	14·6	20				
		2·9	13·8	21				
		1·5	18·2	8				

days just after the normal date of birth, and possibly even before that date, to rule out asphyxia, and even oxygen want, as factors in the complex of reflex and chemical conditions which are involved in the onset of respiratory movements, such as those first observed by Snyder & Rosenfeld (1937). Therefore the statement of those authors, 'Oxygen want depresses and abolishes repiratory movements', may only apply to oxygen want superimposed upon the degree of asphyxia already existing.

Those who regard oxygen want as promoting respiratory movement would only claim that there was a certain optimal degree of anoxia which had that effect; to superimpose a greater degree of anoxia would destroy the respiratory mechanism. So it may be in the case of post-mature foetuses that the degree of anoxia obtaining at about the 32nd day subjects the brain to the atmosphere optimal to the onset of respiratory movement, while a greater degree of anoxia, as Snyder and Rosenfeld found, abolished respiration.

In short, Snyder & Rosenfeld's observations do not seem to us to be inconsistent with the observation of Windle and Buller, Barth and Schultz quoted by Windle (1940) in the following words: 'Atmospheres deficient in oxygen breathed by full term guinea pigs, on which no surgery had been performed and no anaesthetic used, caused rhythmic respiratory movements to start in previously apneic foetuses.' From personal experience one of us (Barcroft) can vouch for the truth of Windle's statement, the difficulty in its interpretation being to be sure that in the guinea-pig so treated the circulation of the mother was sufficiently maintained to insure that the respiratory movements of the foetus were not due to accumulation of CO_2 therein.

The oxygen capacity of the blood rises from the 30th day onwards in the post-mature foetuses. This rise is shown by the blood-gas figures to be in line with the general thesis that the oxygen capacity of the blood responds to the stringency of the conditions to which the organism is exposed. Further experiments are, however, necessary before it can be said that the increased oxygen capacity in these cases is actually caused by the anoxia.

CONCLUSIONS

1. Litters of post-mature foetuses have been produced in rabbits by the injection into the mother of 100 i.u. of chorionic gonadotrophin and 5 mg. of progesterone on the 25th day of pregnancy, confirming the work of Snyder.

2. The foetuses grew on the average from about 45 g. to about 80 g. between the 30th and 36th days. The largest foetus obtained was just over 100 g.

3. The placentas underwent no commensurate alteration in weight. Indeed, it is doubtful whether there was or was not any growth of the foetal placenta after the 31st day.

4. The oxygen saturation of the blood from the posterior fontanelle of the brain fell from the 25th day onwards, and by the 35th day blood from this situation was on the average only about 17% saturated.

5. The brain therefore during the days of post-maturity was subject to an atmosphere which imposed an ever-increasing stringency in respect to oxygen.

6. The active intra-uterine respiratory movements which occurred at this time could reasonably be attributed to the degree of anoxia obtaining, though our experiments do not offer rigid proof that this was the case.

7. It would be reasonable, also, to attribute the ultimate death of the foetuses to asphyxia.

REFERENCES

BARCROFT, J., BARRON, D. H., COWIE, A. T. & FORSHAM, P. H. (1939). *J. Physiol.* **97**, 338.
BARCROFT, J. (1935). *Proc. Roy. Soc.* B, **118**, 242.
BARCROFT *et al.* (1935). *J. Physiol.* **83**, 192.
HAMMOND, J. (1935). *Transactions on the Dynamics of Development*, **10**, 93.
SNYDER, F. F. (1934). *Johns Hopk. Hosp. Bull.* **54**.
SNYDER, F. F. & ROSENFELD, M. (1937). *Amer. J. Physiol.* **119**, 153.
WINDLE, W. F. (1940). *Physiology of the Foetus*, p. 85. Philadelphia and London.